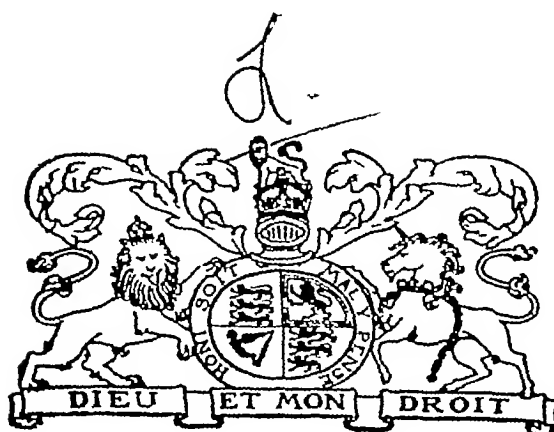




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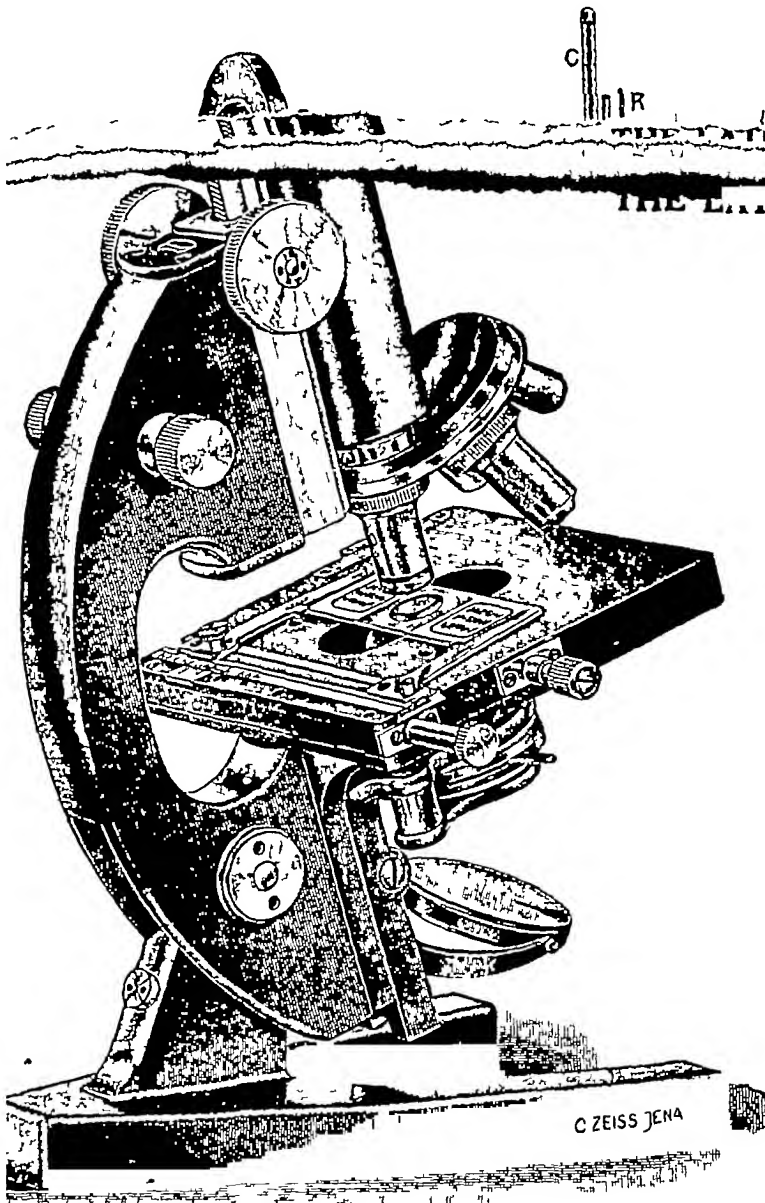
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LIVE.	
CHOPRA, R N, and	
Compounds of Antimony in Leishmaniasis	INCIDENCE OF
CHOPRA, R N, DAVID, J C., and DIKSHIT, B B. A Comparative	
of the Action of Cinchona Alkaloids on the Uterus	
CHOPRA, R N An experimental investigation into the Action of Organic	41
Compounds of Antimony	
CHOPRA, R N, and CHATTERJEE, N R <i>Psoralea corylifolia</i> (Babchi) Its	49
constituents, their Pharmacological Action and Therapeutic Properties	
CHOPRA, R N, and KHEM SINGH GREWAL Opium Habit in India An	57
analysis of 100 cases amongst the Sikh population of Calcutta	
RAY, CHARUBRATA Epidemic Dropsy Its Blood Picture, General and	67
Biochemical	
CUNNINGHAM, J A Simple Method of Handling Squirrels and other Small	81
Animals for Bleeding and other purposes	
CUNNINGHAM, J, NICHOLAS M J, and LAHIRI, B N An Investigation	85
into the Value of an Etherised Vaccine in the Prophylactic Treatment of	
Rabies Part II The action of ether on 'Street Virus' in infected	
Brains	
ACTON, H W, and Bose, J P The Relationship of the colour of rabbits	89
to their susceptibility to Insulin	
ACTON, H W and NAPIER, L EVERARD Post-kala-azar Dermal	97
Leishmaniasis	
BASU, P N Some observations on cases of Anæmia amongst Troops in	107
Bombay	
CAIUS, J F, NAIDU, B P B, and SHAMSHER JANG The Bactericidal	117
action of the Commoner Phenols and of some of their Derivatives on	
<i>Bacillus pestis</i>	
NAIDU, B P B, and SHAMSHER JANG A Note on the Nutrient Broth now	135
used for the culture of <i>Bacillus pestis</i> and its Hydrogen-ion Concentra-	
tion	
VASUDEVAN, A A Case of Sarcosporidial Infection in Man	141
CHANDLER, ASA C The Prevalence and Epidemiology of Hookworm and	143
other Helminthic Infections in India Part X Central India and	
Bombay	
CHANDLER, ASA C The Prevalence and Epidemiology of Hookworm and	159
other Helminthic Infections in India Part XI Madras Presidency	
and Southern States	
NAPIER, L EVERARD The Pentavalent Compounds of Antimony in the	181
Treatment of Kala-azar II No 693 (von Heyden), an analysis of	
the results of the treatment in the first 61 cases	
NAPIER, L EVERARD, and HALDER, K C The cure rate in Kala-azar	187
McCARRISON, R The Experimental production of Stone-in-the-bladder II	197
McCARRISON, R, NEWCOMB, C, VISWANATH, B and NORRIS, R V The	
Relation of Endemic Goitre to the iodine-content of soil and drinking-	
water	207
McCARRISON, R The Experimental production of a new type of Goitre	247
unrelated in its origin to Iodine	
GILL, C A The Forecasting of Malaria Epidemics with special reference	265
to the malaria forecast for the year 1926	

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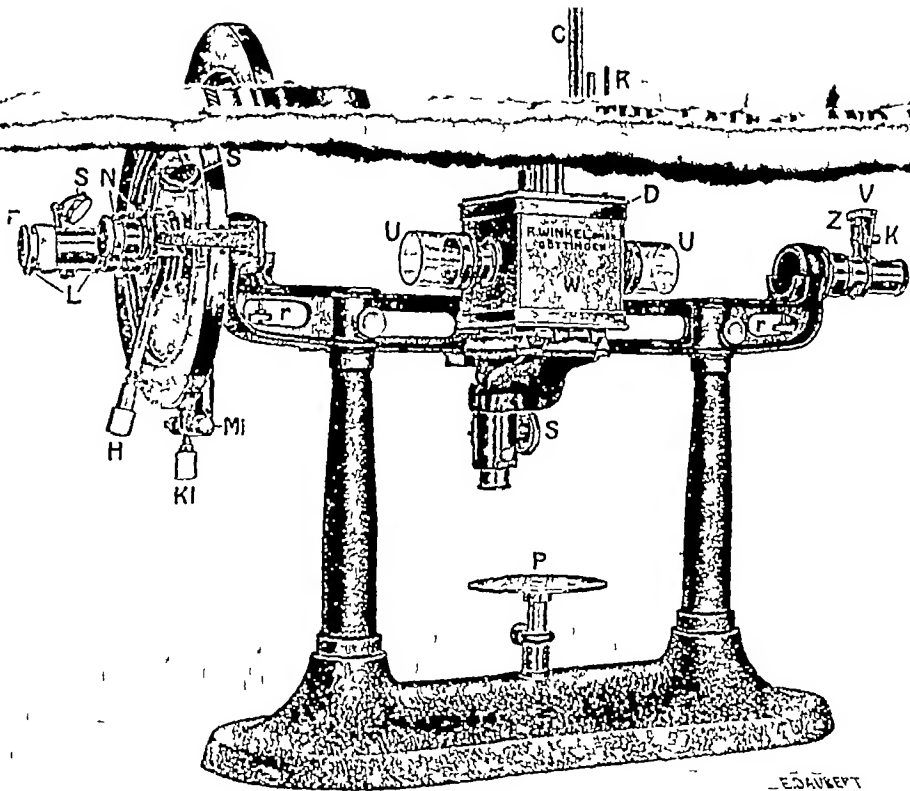
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CHOPRA, R N, and	INCIDENCE OF	
Compounds of Antimony in Leishmaniasis		
CHOPRA, R N, DAVID, J C, and DIKSHIT, B B. A Comparative		
of the Action of Cinchona Alkaloids on the Uterus		
Buccal Cavity and Classification of Anopheline Mosquitoes		301
COVELL, G, and BAILY, J D. Observations on Malaria in the Andamans,		
with special reference to the Enlarged Spleen in Adults		309
CAIUS, J F, KAMAT, S A, and NAIDU, B P B. The Bactericidal Action		
of some Organic Compounds of Mercury on <i>Bacillus pestis</i>		327
NAIDU, B P B, and SHAMSHER JANG. Production of Alkalinity by		
<i>B. pestis</i> in Broth and the Effect of this Alkalinity on the Toxicity		
and Potency of the Prophylactic		335
CHOPRA, R N, and DAVID, J C. The Pharmacological Action of		
Quinamine		343
ACTON, HUGH W. Porokeratosis its Causation and Treatment		349
ACTON, HUGH W. Acquired Angiomata or Sarcoids		355
BRAHMACHARI, B B. On the Prevalence of <i>Vibrio cholerae</i> in Some of		
the Endemic Areas of Bengal		361
CUNNINGHAM, J. A Note on the Degeneration of Vaccine Lymph on		
Passage through the same Vaccinifer		373
STRICKLAND, C, and CHOWDHURY, K L. An Anopheline Survey of the		
Bengal Districts		377
HUGHES, T A, and SHRIVASTAVA, D L. Observations on Tropical Cirrhosis		
of the Liver with Special Reference to the Fragility of the Red Blood		
Cells		427
HUGHES, T A, and SHRIVASTAVA, D L. Carbohydrate Tolerance in two		
Cases of Muscular Dystrophy		437
HUGHES, T A, and SHRIVASTAVA, D L. A Study of Renal Oedema		445
McCOMBIE YOUNG, T C. A Field Study of Lathyrism		453
NAPIER, L EVERARD. The Infectivity of the Flagellate Form of <i>Leishmania</i>		
<i>donovani</i>		481
McCARRISON, R. The Experimental Prevention of Stone-in-the-bladder in		
Rats III		485
DAS GUPTA, B M. Note on <i>Trichomonas</i> found in the Gut of a Non-		
poisonous Colubrine Snake		489
HODGSON, E C, VARDON, A C, and ZORAWAR SINGH. Studies of the		
Effects of Antimony Salts No I. The Effect of Antimony Salts on		
Conception and Pregnancy in Animals		491
MUIR, E. The Effect of Kala-azar on Leprosy		497
MUIR, E. Sodium Hydnocarpate in Leprosy, Suggested Improvements in		
Administration		501
MUIR, E. The Iodide-Antimony Treatment of Leprosy		507
PURI, I M. A note on the full-grown Larvæ of <i>Anopheles jamesi</i>		
Theobald, <i>A. fuliginosus</i> Giles, <i>A. pallidus</i> Theobald and <i>A. ramsayi</i>		
Covell (Culicidæ, Diptera)		511
BARRAUD, P J. A Note on Rat Fleas collected in Assam		519
GREVAL, S D S. On Wassermann Reaction I. Effect of an excess of		
the hæmolytic amboceptor on the M H D of the complement		523
SHORTT, H E, SRIBAS DAS, and CHIRANJI LAL. The Finding of Parasites		
in the Peripheral Blood of Kala-azar Cases by direct Microscopical		
Examination		529
SHORTT, H E, and SWAMINATH, C S. <i>Monocystis mackiei</i> n. sp. parasitic		
in <i>Phlebotomus argentipes</i> , Ann and Brun		539



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CHOPRA, R N, and	INCIDENCE OF	
Compounds of Antimony in Leishmaniasis		
CHOPRA, R N, DAVID, J C, and DIKSHIT, B B	A Comparative	
of the Action of Cinchona Alkaloids on the Uterus		
SEN, K C, and BASU, S K	On the Hæmolysis of Red Blood Corpuscles	
by Bile Salts and the Inhibiting Action of Normal Serum and Egg		
Albumin, with reference to the Permeability Change of the Stroma		581
SINTON, J A	Notes on some Indian Species of the Genus <i>phlebotomus</i>	
Part XXII The female of <i>P. newstead</i> Sinton, 1926		589
SINTON, J A, BIRD, W, and EATE, S N	Studies in Malaria with Special	
Reference to Treatment Part VIII The oral administration of		
Quinine-stovarsol in the treatment of chronic benign tertian malaria		595
MANIOLD, J A, and DEMONTE, A J	Report on an Investigation of	
Dysentery and Diarrhoea in Poona		601
BOYD, T C and ROY, A C	Notes on the Cholesterol Content of Indian	
Blood in Health and in Leprosy		643
BARRAUD, P J	A Revision of the Culicine Mosquitoes of India	
Part XXIII The genus <i>Aedes</i> (sens lat) and the classification of		
the subgenera. Descriptions of the Indian species of <i>Aedes</i> ( <i>Aedimor-</i>		
<i>phus</i> ), <i>Aedes</i> ( <i>Ochlerotatus</i> ), and <i>Aedes</i> ( <i>Banksinella</i> ), with notes on		
<i>Aedes</i> ( <i>Stegomyia</i> ) <i>variegatus</i>		653
BARRAUD, P J, and COVELL, G	The Morphology of the Buccal Cavity in	
Anopheline and Culicine Mosquitoes		671
GREVAL, S D S	<i>Apropos</i> of Studies in Syphilis in Rabbits Rabbits	
naturally positive to Wassermann and Kahn		681
GREVAL, S D S	Kahn, Microkahn and Wassermann in Leprosy	683
STEWART, A D, and BANERJEE, N L	Comparative Observations on the	
Wijs' and Hanus' Methods of Determining the Iodine Values of Oils		687
CHANDLER, ASA C	The Prevalence and Epidemiology of Hookworm and	
other Helminthic Infections in India Part XII General Summary		
and Conclusions		695
MCCOMBIE YOUNG, T C, and BAILY, J D	Malaria in Coorg	745
MCCARRISON, R	Studies on Lathyrism II	797
MCCARRISON, R	The Experimental Production of Stone-in-the-bladder. IV	801
MUIR, E, and HENDERSON, J M	Rat Leprosy A Record of Experimental	
Work carried on at the School of Tropical Medicine and Hygiene,		
Calcutta, between October 1925 and August 1927		807
CUNNINGHAM, J, and MHASKAR, K S	The Hydrogen-ion Content of	
Vaccine Lymph and its Relation to the Potency and Keeping Properties		
of Lymph prepared under tropical conditions		819
SHORTT, H E, and SWAMINATH, C S	The Method of feeding of <i>Phlebo-</i>	
<i>tomus argentipes</i> with Relation to its bearing on the Transmission of		
Kala-azar		827

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# SOME FACTORS WHICH INFLUENCE THE INCIDENCE OF LEPROSY

BY

E MUIR,

*Research Worker in Leprosy under the Indian Research Fund Association*

[Received for publication, January 27, 1927]

## INTRODUCTORY

IN an article entitled 'The World Incidence of Leprosy in Relation to Meteorological Conditions' published in 1923 Sir Leonard Rogers shows the importance of a high rainfall and humidity in increasing the incidence of leprosy, and this point is well brought out in the map of the world and special maps of India which he appends. He also says 'Elevation above sea level and geological formation appear to be subsidiary factors influencing leprosy incidence.'

In the present paper I have tried to survey in more detail the various factors which play a part, and have found that, while the study of large areas such as countries and provinces, between which there are very great variations in humidity, seems to emphasise the importance of humidity, other factors, such as diet, race, caste and soil, appear to influence the incidence more markedly when smaller areas, such as districts and thanas, are considered. The mode of infection has not been discussed but only conditions which lead to the lowering of the resistance of those who are exposed to infection. The greater the lowering of resistance the greater is the probability of the disease being of an acute type, and such cases are the more likely to become highly infectious and spread the disease to those who come into contact with them especially if that contact be close and prolonged.

## METHOD OF INVESTIGATION

The 1921 census gives the number of lepers in India as 102,513. It is not stated that this is the total number of those suffering from this disease, and, indeed, the way in which these census figures are collected makes it clear that this is not so. The enumerators who collected the returns are not medical men, nor have they had any training in making a diagnosis. The census officer for Bengal makes the following statement —

'The medical man who is conversant with the manifestations of the disease can diagnose leprosy before the ulcerous stage, by the form of the patches and swellings which appear, but it would have been quite impossible to expect the census enumerators to make such a diagnosis even if they were



actually to examine all the persons they were to enumerate, which, of course, they do not do. They were therefore required only to record as lepers those in whom the disease had reached the ulcerous stage.

As those who have reached this state constitute only a small minority of those suffering from leprosy in any endemic area, the census figures cannot be expected to give any clear idea of the actual total numbers.

Moreover, there are two reasons which tend to falsify the figures—

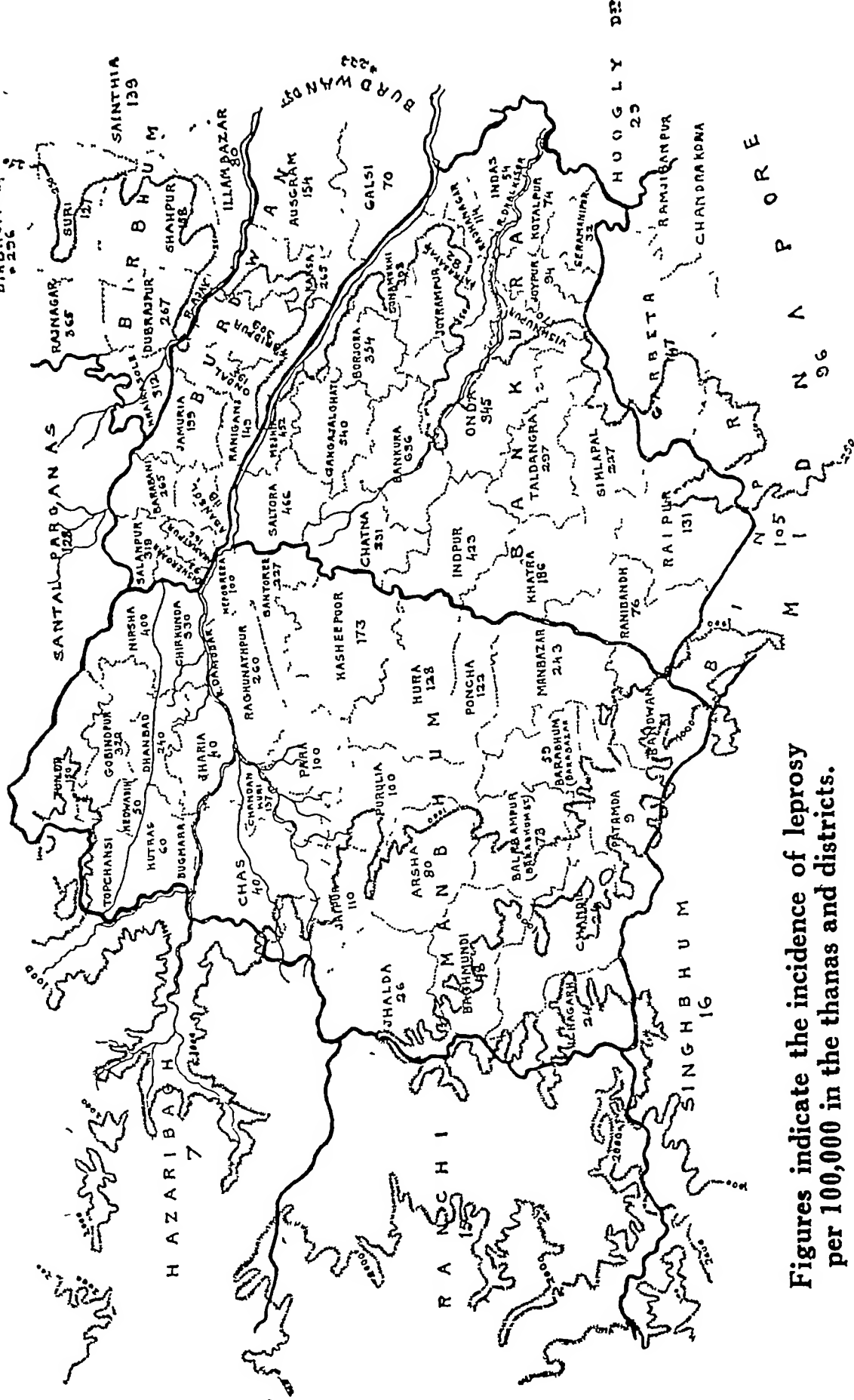
(1) The slow, insidious onset of leprosy, the absence of pain and the fact that many patients are ignorant that they are suffering from leprosy until the disease has advanced far beyond the early stage. As an example of this it may be mentioned that, of 60 menials in the employ of a well known institution in Calcutta, five were found to have early leprosy, although they were ignorant of it.

(2) The desire which patients have to hide the fact that they are suffering from leprosy. This desire is very natural as they fear that, if the nature of their disease is known, they may lose their employment or be socially ostracised. As an example of this may be mentioned the fact that of the leper patients who were attending our leper clinic in Calcutta and who were necessarily all aware that they were suffering from leprosy, only 7 per cent had their names entered as lepers in the 1921 census returns.

As the census obviously and avowedly fails to give the total number of lepers, can it be expected, at least in a broad sense, to give indications of the relative incidence of leprosy in different areas? This implies (a) the assumption that the number of obvious lepers bears approximately a fixed relation to the actual total number, (b) that the enumerators in the different areas are on an average equally skilful and reliable, and (c) that the desire on the part of the affected to hide the disease is not greater in one area than in another. These are rather large assumptions, but when the census figures are examined critically there is clear evidence that the numbers, while necessarily inaccurate, do give a useful indication of the relative incidence of leprosy. It would be expected that members of the better educated and cultured castes would have more reason to hide their disease than the less enlightened yet in some areas the incidence of leprosy is given as high among all castes and in others as low in them all. This shows that while the desire to hide the disease may vary among different classes, the variation is not great enough to cover the difference of the geographical incidence. Another evidence of relative accuracy of the statistics is shown by reference to Map 1 which shows that in neighbouring thanas the figures are approximately equal and the numbers only gradually increase or diminish as one passes to east or west.

Assuming, therefore, the usefulness of the census figures as indicating the relative incidence of leprosy in different zones, one of the most highly endemic areas was selected along with the less affected neighbouring districts in order to try to discover by comparison of conditions the factors which govern the variation. The principles discovered by this comparison were then tested as to their applicability in other parts of India.

# Map 1.

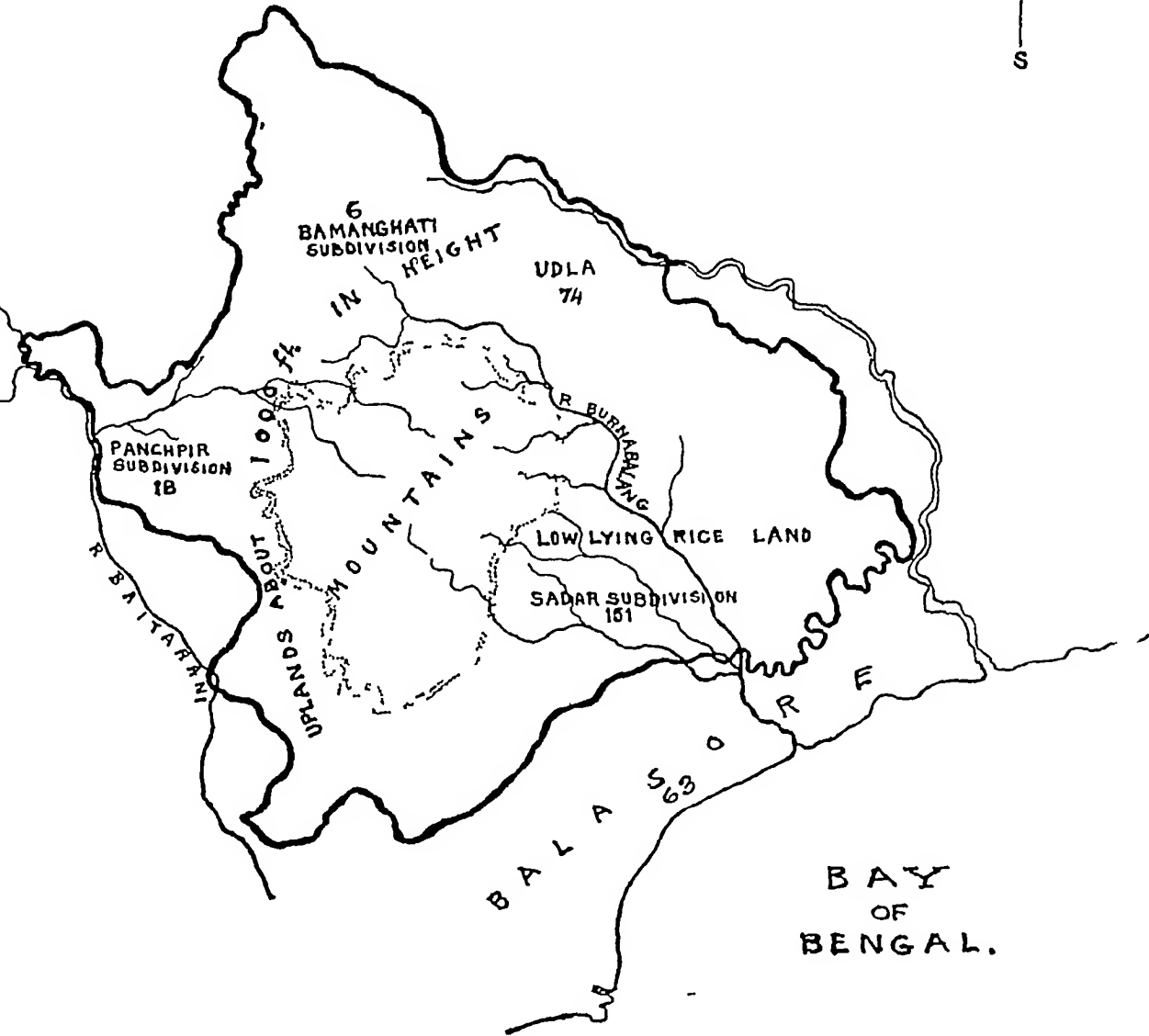
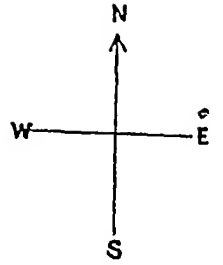


**Figures indicate the incidence of leprosy per 100,000 in the thanas and districts.**

MAP 2

The figures indicate the number  
of lepers per 100,000 in each  
sub-division.

MAYURBHUNJ.



## INVESTIGATION IN BINGAL-BIHAR ZONE

The area selected consists of—

- (1) The plateau which extends, more or less irregularly, westwards across India from Ranchi and Hazaribagh
- (2) The sloping land lying to the north and east which leads down from this plateau to the plains
- (3) The Gangetic valley

It was found that in the first of these areas the incidence of leprosy was very low, in the third it was somewhat higher, while in the second or intermediate area it was very high indeed. This will be seen by examining Table I, and in more detail in Map 1.

Further study of this map shows that leprosy is very rife in the western parts of the Birbhum, Burdwan, Bankura and Midnapur districts, while the incidence is much less in the eastern parts of these districts. This is most markedly seen in the district of Bankura. In the Western or Sadar sub-division of this district the number of lepers given is 4,364, whereas in the Bishnupur sub-division it is only 928.

Taking the Manbhum district, which lies to the west of the Bankura district, we see the same relationship reversed, the eastern half being the more leprous. If a line be drawn through the middle of the Manbhum district from north to south parallel to the contours of the principal elevations in the country, the statistics give the following —

TABLE I

	East of the line	West of the line
Population	906,650	641,128
Number of lepers	1,604	327
Rate of lepers per 100,000	176	55

While in the district of Ranchi, which lies west of the Manbhum district, the number of lepers per 100,000 is only 7.6.

Another illustration of this variation in the incidence of leprosy is found in the Mayurbhunj State (Map 2), which lies to the south of the country just described. This State has a core of mountains occupying the centre. To the south-east is a comparatively low-lying tract bordering on the district of Balasore, while to the north and west the land is high, varying in elevation from 500 to well over 1,000 feet.

Mr Philip, Political Agent of the Orissa States, kindly had collected for me the leprosy statistics of Mayurbhunj. Leprosy was found in 151 per 100,000 in the Sadar sub-division, which is a low-lying, rice-growing region with fish-bearing streams bordering on the Balasore district where the census returns give leprosy as 63 per 100,000. In the Udla sub-division, which is partly low-lying and similar to the Sadar sub-division, but also partly high land, there were 74 per 100,000. In the Panchpir sub-division which lies on higher land and in which the streams are on a higher level and do not harbour fish, the incidence is given as 18 per 100,000, in Bamanghatti the highest and least watered by perennial, fish-bearing, streams, the incidence is only 6 per 100,000.

Mr Philip adds that, while in the whole State the Sonthals are given as having the largest incidence of leprosy, 149 out of 598 or about a quarter of the whole, yet in Bamanghatti, the sub-division in which we get the lowest incidence of leprosy, the population of Sonthals predominates

The study of the above areas therefore showed that a line which passes from north to south between the Birbhum, Burdwan, Bankura and Midnapore districts to the east and the Sonthal Parganas and Manbhum district to the west and then follows a southern course through the east of the Mayurbhunj State, passes through an area of maximum leprosy incidence, the disease diminishing more or less gradually to the east and west of this line. With this as a starting point the question was considered as to why leprosy should be so prevalent along this line.

If we take one of the most highly endemic areas, which has its centre in the thana of Bankura with 636 per 100,000, and compare it with the less leprous areas lying to the east and west, what are the differences to be found which will account for the greater amount of leprosy in the central area? Bankura is well known as a famine area, the Western, Sadar sub-division being most affected. The frequent famines are due to the nature of the land and of the soil. The land is chiefly of a sloping nature with an inclination towards the east, so that, though the rainfall is not appreciably less than in the eastern sub-division, the water tends to run off and wash away much of the surface soil. The soil is laterite and very porous, so that rain water soaks rapidly into the ground and is not available for the growth of crops. In consequence of the sloping nature of the land and the porous nature of the soil, large areas of land are unable to yield crops except in the rainy season, and even the main crop, that of rice, is precariously dependent upon a steady fall of rain in the monsoons. Fodder for cattle is scarce for the same reason, and, though large herds of cattle are to be seen in the villages, they are small in build and are kept mainly for their dung which is used for fuel, the yield of milk being small or nil. To the east the land is flat and of a clayey, less porous nature, where better crops can be raised and where the water supply is less precarious. To the west in western Manbhum we have more clay and the subsoil water level is higher, and in the flat plateau land better crops are raised.

The main difference then that we find between the central, highly leprous zone and the less endemic areas to the east and west is one of diet, dependent in the central area on the poor and irregular nature of the crops available for human consumption and for fodder for cattle, which conditions again are dependent on the physical features of the country and the nature of the soil. In the central area the diet of the people is limited to rice and the less digestible forms of 'dal', milk and fresh vegetables are available only for the rich. The poor are seldom out of debt due to the poverty in which they are left by successive famine years. The lack of animal food is made up for in many places by the consumption of badly preserved or decomposing fish. Strange though it may seem, people belonging to castes which have been more recently absorbed from aboriginalism into Hinduism eat the

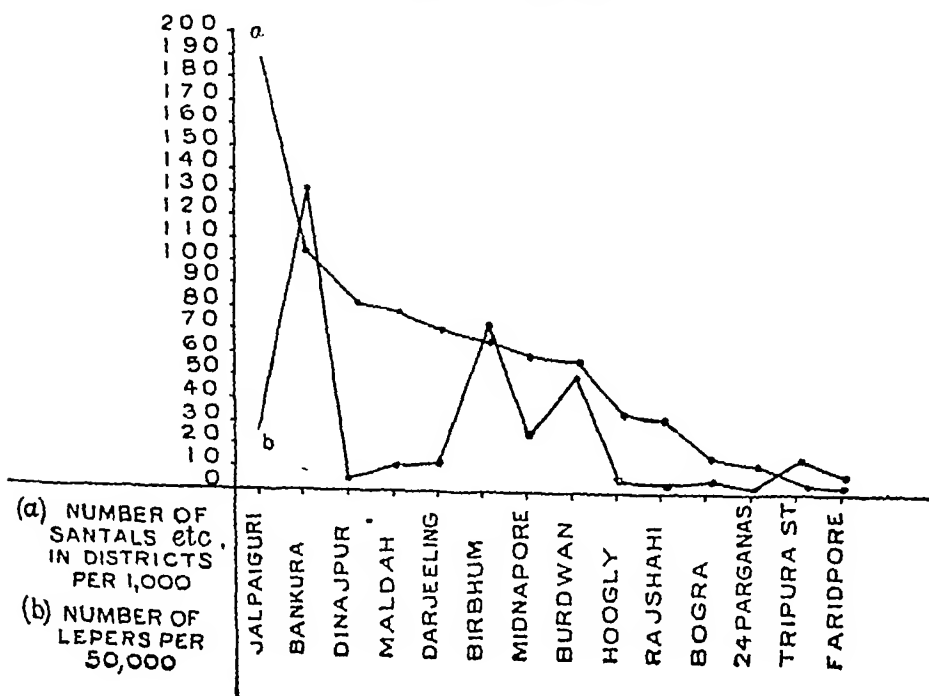
carcasses of cattle which die from various diseases, the meat being to a greater or less extent decomposed. Not only is decomposing meat and fish eaten but carbo-hydrate food is often preferred in a state of decomposition, rice is cooked at night and left overnight soaking in water, so that when it is eaten in the middle of the following day, it has decomposed to a certain extent. In certain areas a still further stage of decomposition is arrived at, rice beer being made and drunk in large quantities by the people.

On the western plateau better crops and fodder for cattle are available, the crops are less precarious, fish are not obtainable and the diet of the people is more nutritious, while the temptation to eat decomposed meat or carbo-hydrates does not exist as the food is much more tasty and its composition is better balanced. In the Gangetic valley fish is eaten by the people, decomposed fish is not eaten, (1) because it is always procurable fresh, and (2) because the greater variety of food available on the more fertile plains does not tempt even the poor to seek the tastiness imparted by decomposing food.

Doubtless there is a certain amount of exemption from leprosy due to the low humidity and lower temperature of the Ranchi-Hazaribagh plateau, but the same cannot be said for the difference between the plains of the Gangetic valley and the slopes of east Manbhum and west Bankura.

Another factor which it was considered might affect the incidence of leprosy was the various races and castes which inhabit different areas. Graph I prepared from the Bengal census returns shows a certain rough degree of correlation between the incidence of leprosy and the population of three aboriginal races, the Sonthals, Mundas and Gonds. But when the

GRAPH I.  
LEPROSY AND HUMIDITY.



'depressed classes' (including Bagdis, Bowris, Sonthals, Munchis, Kurmis, Chasi Kaibaitas, Namasudras, Rajbanshis) were taken (Graph II), there was no apparent correlation with the leprosy curve Table II, however, brings

TABLE II	
Caste	Caste incidence in Bihar and Orissa Number per 100,000
Bowri	145
Chasa	74
Khandit	59
Bhuyan	46
Teli	43
Kurmi	39
Tanti	34
Sonthal	29
Brahman	28

out interesting results with regard to the extraordinary prevalence of leprosy among the Bowri caste in Bihar who are notorious eaters of carrion. We shall discuss the bearing of caste and race more fully later.

Furthermore, when we examine the effects of humidity in Bengal we find the same lack of correlation, as is shown by Graph III.

#### INVESTIGATION IN OTHER ZONES

The result then of the study of this limited area points to the main factors in a higher incidence of leprosy being famine or semi-famine conditions, the eating of decomposing food such as carrion, rotten or badly preserved fish and decomposing rice. The eating of this fish was found to be connected with the nature of the soil and with the habits of certain races or castes of people, while humidity and rainfall, in this area at least, were not found to be important.

An attempt was then made to test these factors as to their influence on the high incidence of leprosy in other parts of India.

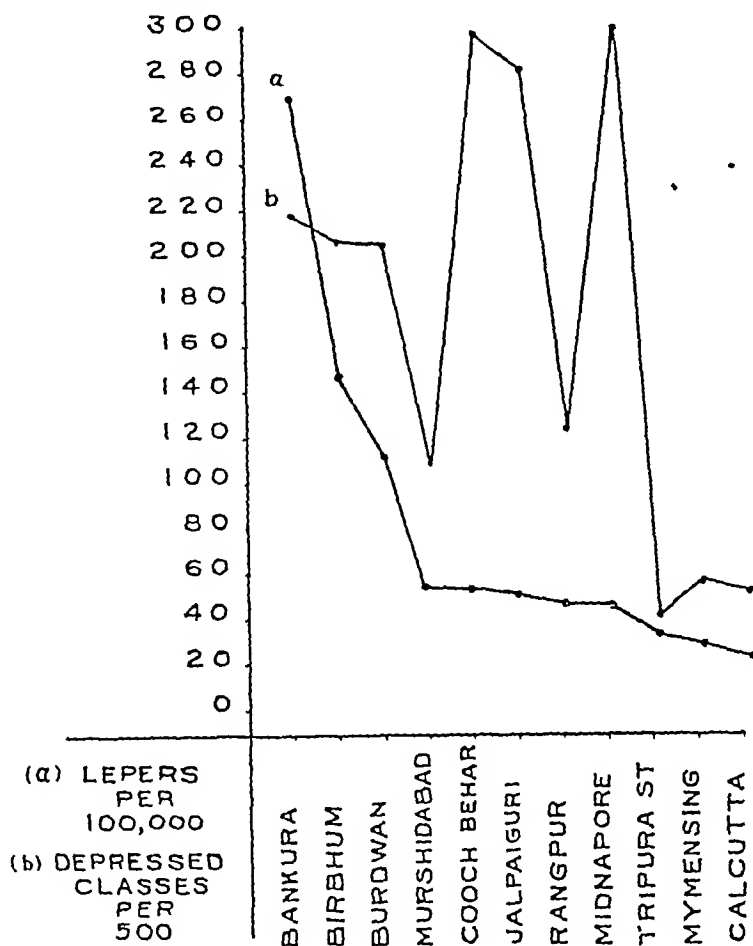
Taking the hill country lying between Assam and Burmah, we find the following contrasts between the different districts —

TABLE III	
District	Lepers per 100,000
Chin Hills	212
Lushai Hills	6
Naga Hills	80
Hill district of Arakan	397
Pakollu Hill tracts	54

The greatest contrast is that between the Chin Hills and the Lushai Hills, which lie alongside each other. The most obvious difference between these two peoples is that of their habits with regard to diet. In contrast to the more natural diet of the Lushais, in the Chin Hills decomposing or badly preserved fish is a special delicacy, and one of the most important articles of diet, while in the Arakan Hills, this is even more so. In many of the districts of Burmah the people, being Buddhists, are averse to taking life though fond of eating flesh and fish. The tendency therefore is to eat the

flesh of animals which have died a natural death, the meat of which has necessarily begun to decompose, and fish which has been caught by others and brought from a distance and therefore is not fresh

GRAPH II  
LEPROSY AND DEPRESSED CLASSES.



Sir J Hutchinson points out that leprosy used to be more common among Roman Catholics than among those of other creeds because of the fish-eating rules of the former community, fish often being eaten preserved, and that badly, as it could not be obtained fresh. In Burmah we seem to have another and even more obvious example of how the perversion of the tenets of a great religion may lead to the consumption of decomposing proteins and consequently to a high incidence of leprosy.

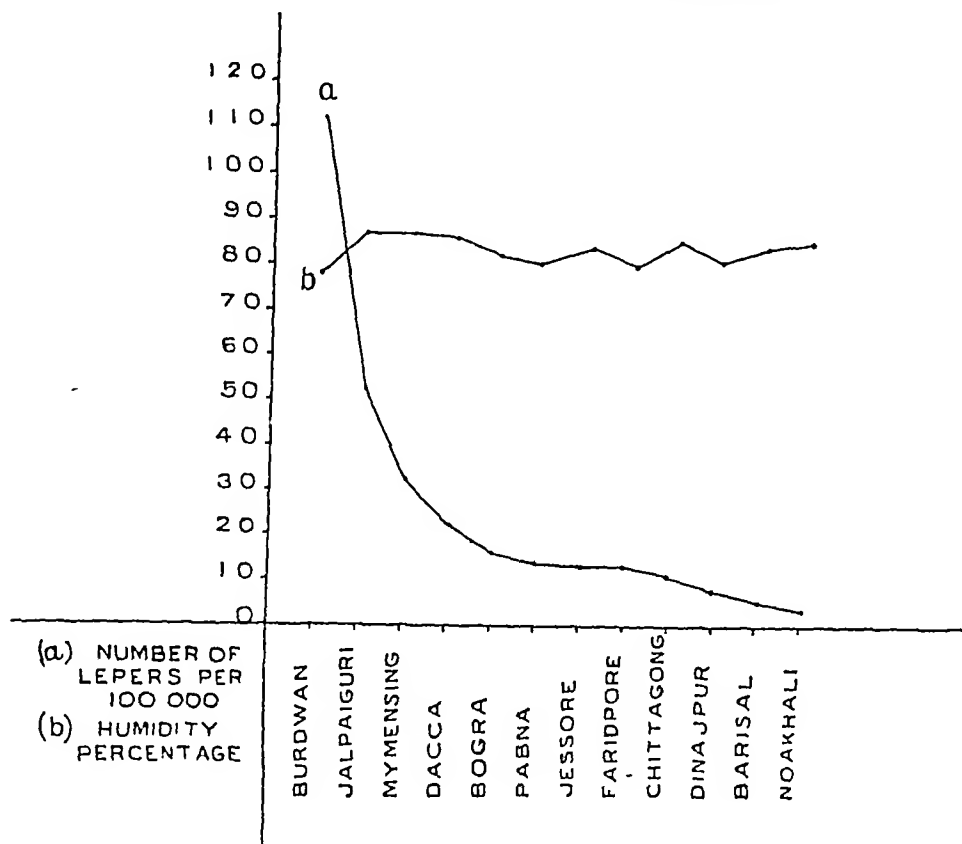
If again we take the Bombay presidency, we find that the highest incidence of leprosy centres in the Satara district with 129 and in East Khandesh with 102 per 100,000. The latter corresponds to the neighbouring highly endemic area in the Central Provinces. The former is a country over 2,000 feet above sea-level with hills rising to 3,500 feet in its western part.

Dr D V Kelker has kindly sent me some interesting information regarding this area collected from the Collector of Satara and the Assistant Collector of south Satara.



'Leprosy in this district is concentrated in Patna Taluka, and particularly in the wild, hilly, western portion of that Taluka. Particularly villages to the west of the Koyna and Moyna valleys afford a vast field for investigation

GRAPH III  
**LEPROSY AND ABORIGINALS.**



As regards the causes of leprosy in this tract, many of the people are very poor, and probably ill-nourished. It is possible that they are of an older and more aboriginal stock than the Hindus of the rest of the district. They certainly appear to be. The annual rainfall is excessive, at Helvak it averages 250 inches. Dried fish is imported from the Kumbharly pass and by the time it is eaten by the people of these villages it is often stinking and putrescent. Bad fish has for generations been considered to be a cause of leprosy.

In south Satara 44 per cent of the villages are leprosy, and the number of lepers per 100,000 given in the census is 200.

In another letter he states —

'The parts most affected are those lying on the hill sides which form arms or extensions of arms of the Western Ghat. The rainfall is usually heavy and is only useful in denuding the soil, the soil surface that is washed away is deposited at places some scores of miles away. Thus the soil affords only a poor kind of grain for the people and their physique is still more eaten up by the ravages of malaria and other epidemics.'

The incidence of leprosy in the Central Provinces is worthy of notice and similar conditions are found to govern its distribution there. (See



in these northern districts and forms a more nourishing diet than millet. There is also fodder for the cattle and milk forms a large part of the diet of the people.

In the plains of the Punjab leprosy is uncommon and there we have the absence of famine conditions, and the people have milk, wheat and vegetables as their principal diet.

### DISCUSSION

At first sight it would appear from what we have related above that the factors which determine the incidence of leprosy are very complicated indeed. Rainfall, humidity, elevation, slope of land and porosity of soil, race and caste, poverty, famine succeeding years of comparatively good crops, the habit of eating decomposing food.

Is there any relationship among these various factors or can we correlate them in any way?

(1) *Diet*—The most outstanding determinant would appear to be dietetic, and there are two main elements in this—

(a) Starvation due to a deficiency of certain food essentials

(b) Intoxication due to decomposing food

In most instances these two are combined, the one leading to the other. If normal proteins are lacking in the diet we can only expect that nature should demand proteins in whatever form they can be had, and hence the eating of decomposing fish and meat. The eating of decomposing rice and other cereals would appear to be due to a similar cause. To begin with, rice is cooked only once a day to save the time required for a second cooking, but later stale rice is preferred and is kept in water so as to accelerate decomposition. It may even be kept over another period of 24 hours so as to allow it to putrefy still more, and this process is often prolonged still further to form rice beer. In eastern Orissa such decomposed rice is said to be taken as it acts not only as food but to a certain extent as a narcotic enabling people to forget their hunger and their woes.

In Calcutta a seer of dried prawns may be bought for Re 1. It will provide tasty food and a sufficiency of proteins for a month and such delicacies can be had still more cheaply in the neighbourhood of the places where the fish are caught and prepared. So there is little wonder that food of this kind is sought and appreciated in regions where the crops are poor and lacking in relish and nourishment.

In Burmah 'Ngapi,' a decomposing fish concoction, is a much sought-after delicacy. In the Naga and Garo Hills and among the hill tribes of Manipur small dried fish are imperfectly preserved and eaten. In Satara decomposing fish is brought up from the coast and supplies the wants of the people where the soil is poor and the food is not nourishing. Another reason for preserving fish instead of eating it fresh is that it can be caught in the streams only in certain seasons especially in the rains when fish come up in abundance. A supply is caught at that time sufficient to last for the whole year. In the plains of Bengal, where fresh fish can be obtained all

the year round from rivers and tanks, preserved fish is not eaten, and leprosy is less common

It may be asked what is the connection between rotten fish and leprosy? Does the fish convey the infection? There is no reason to believe that this is so. But any one who has had prolonged experience of the treatment of leprosy must be aware to what extent the disease is influenced by diet, the eating of bad food often being sufficient to bring on a severe reaction. Starvation also, by lowering the resistance, lays the patient open to an attack. In fact there is every reason to believe that the teaching of Sir J. Hutchinson with regard to decomposing fish and meat as causing leprosy is substantially true, and it is to be regretted that the emphasis he laid on this to the exclusion of other causes brought discredit on his theory, when it could be proved for instance that leprosy was not uncommon among the strictly vegetarian Marwaris.

Another dietetic factor of importance is the absence of milk from the diet. In Burmah milk is seldom drunk as is also the case in China. Indeed, eating of bad fish among the Chinese and allied races seems to take the place which milk and dairy produce take in the more fertile parts of India. As has been mentioned above, the lack of fodder due to poor soil also results in the absence of milk from the diet. In several places such as Berar and East Khandesh buffaloes are kept instead of cows, and their milk yields such a high percentage of cream that ghee is made and sold in the large cities, milk and its products thus being cut out of the diet of the ryot.

If we accept diet then as the principal cause in determining a high incidence of leprosy we shall be in a position to consider the bearing which other factors mentioned above have upon the disease.

(2) *Race, Caste and Religion*—As has been mentioned above, leprosy is exceedingly common among the Chinese. In China itself it is most common in the south with Canton as the centre, and it is the Cantonese who are the great emigrants from China. In the Strait Settlements and Federated Malay States leprosy is far more common among the Chinese in proportion to their numbers than among any other race and the same is said to hold good in Burmah. Even in Calcutta the frequency of leprosy among Chinese, as witnessed by the number of them who attend our leprosy clinic, is very noteworthy, and yet the Chinese of Calcutta are a remarkably hard working, temperate and cleanly race. The most probable explanation lies in the absence of milk from their diet and the substitution of rotten fish, and there is nothing more remarkable than the speed with which our Chinese out-patients invariably recover, as compared with the Indian patients attending the same dispensary, when the diet is changed to milk, whole-wheat bread and fresh vegetables. The effect of Buddhism on those who have been brought up in the habits of a more primitive religion has also been mentioned, the objection to destroying life leading to indulgence in decomposing flesh and fish.

It has often been pointed out that primitive peoples have more leprosy than the higher and more civilised. This statement should, however, be

qualified Graph I shows that there is no close correlation between the incidence of leprosy and the aboriginal races of Bengal, and Graph II shows an even more marked lack of correlation with the depressed castes. In Bihar and Orissa Table II shows a very marked preponderance of leprosy in one particular caste, the Bowris, who show 145 per 100,000 suffering from leprosy, while the most typically aboriginal race, the Sonthals, shows 29 which compares favourably with the incidence among the highest caste, the Brahmin with 28 per 100,000. The Bowris are well known to be unclean eaters. They indulge in the eating of decomposing carcasses and the various stages of stale rice including rice beer to a far greater extent than the other castes of the province.

It has also been shown, as in the case of the Sonthals of Mayurbhunj, that aboriginals living in their upland, natural surroundings harbour but little leprosy, but, when they come down to the plains to work, and come in contact with a higher (?) civilization, they are apt to fall a prey to the dietetic and other changes which lay them open to the attack of leprosy.

If we think of the history of many of these depressed tribes and castes we can see a reason for their location in places where the soil is so poor. They represent the aboriginal inhabitants to a large extent, and were driven from the more fertile plains by the more powerful invaders and took refuge in the more hilly parts where they lived in the jungle. Those of them who took to agriculture had to be content with poor land to till. Many would be reduced to serfdom by getting into debt to money lenders and others. Those of them who live under more natural conditions as in Hazaribagh and Ranchi and west Mayurbhunj escape leprosy, but the incidence is high among those whom starvation drives to eating decomposed food. Sexual promiscuousness and venereal disease, which are very common among certain castes, are also very important predisposing causes. Leprosy is doubtless spread from highly endemic to less endemic areas by some of the factors mentioned above. Thus famine years drive the inhabitants of these leprous areas to the more fertile and less leprous districts, where they act as farm and industrial labourers or as house servants. Those of them who are suffering from slight degrees of leprous infection tend to become worse and spread the infection in the area to which they have gone.

(3) *Soil conditions and the physical features of the land*—These are also accountable for starvation as has been mentioned above. In places where the soil is fertile and yields good crops year after year leprosy is rare. Where, however, the soil is not rich, the population is small and proportionate to the number it is able to support. In zones in which the land is excessively porous, though reasonably fertile, and gives one good staple food crop (as rice or millet), in the year, we tend to get a large population living under famine conditions. They have gradually become accustomed to supplement their diet in famine years by recourse to such putrefying food, both protein and carbo-hydrate, as they can obtain, and it is among such people that leprosy is common. Certain laterite and black

cotton soils seem to be those which most commonly lead to this. These conditions are accentuated when the sloping nature of the ground leads to the washing away of the surface soil in the form of silt.

(4) *Humidity and rainfall*—There is no marked correlation between humidity and leprosy in the areas studied, but rainfall may have a more marked effect in various indirect ways. When in excess it may wash away upper soil and impoverish the ground for crops, thus leading to starvation, or, it may lead to malaria which weakens the resistance of the inhabitants to leprosy. When the soil is excessively porous even a high rainfall may be associated with drought. Doubtless excessive moisture accompanied by a high temperature by lowering the resistance predisposes to leprosy as to other diseases, but as far as this study has gone the correlation between leprosy and humidity does not appear to be close.

#### SUGGESTIONS

(a) It would appear from the above discussion that a disease like leprosy, which is dependent to such a great extent on the economic conditions of the people, must wait for its elimination until such conditions are improved, and it is likely to linger on in famine areas and among backward tribes and castes even after it has been got rid of in more fertile and enlightened zones. Moreover, such leprous areas are likely to continue to be sources of infection. This should be a potent reason for agricultural reform in these districts and for efforts to improve the condition of the backward classes of the community.

(b) In certain leper asylums the diet is altogether insufficient and is calculated to cause the exacerbation of the disease. In one institution for instance, rice is given along with 6 pice a day. The patients in such circumstances, unless they are able to supplement their allowance in some way or other, are driven to the alternatives of starvation if they wait to get good food, or suffering from the poisonous effects of bad food if they eat what is provided. No good results can be expected from special treatment in such an institution.

In other asylums, however, the patients are employed in gardening, farming and other pursuits which make it possible for them to supplement their diet by the work of their own hands.

(c) This paper gives only some suggestions as to the chief causes which predispose to leprosy. It does not deal at all with the ways in which infection takes place. The writer will be glad to receive comments from readers who are acquainted with the conditions in different endemic and non-endemic areas, which may either confirm or modify the views given above.

Leprosy, as it exists in India, is a disease about which very little is known and any reliable information bearing upon the subject will be welcomed.

(d) Although conclusions based upon statistics given by the census report are of value, the results must be tested by a careful survey carried out by experts to show —

- (1) What is the ratio of the actual number of lepers to the 1921 census
- (2) What are the actual factors which determine high endemicity in each area

Selected highly endemic areas should be examined and neighbouring areas of low endemicity compared to bring out the contrasting conditions

Leprosy, as it is found in towns, is no index of the actual endemicity of a district or province, as economic and other conditions attract lepers to cities for work or for begging purposes from quite a long distance

A large number of those suffering from leprosy in Calcutta, though perhaps previously infected, developed the disease while working in the city due to the unhygienic conditions under which they live. Many of these people come from Bihar and Orissa and the United Provinces. In Bombay in the same way large numbers are attracted from Satara, Khandesh and Berar

Provision must be made in these cities for the treatment of these lepers. But the problem is primarily a rural one. This paper shows that the distribution of leprosy is very patchy. One taluka may have a very large number while a neighbouring taluka has very few or none at all. If the campaign against leprosy is to be intelligently and economically carried out it is necessary first of all to have clear indications as to where the strongholds of the disease lie and what are its allies in the form of climatic and soil conditions, dietary errors, accompanying diseases, social and racial habits, etc.

In carrying out such a survey it is not necessary to gather statistics as to the actual number of lepers all over the country. It should be sufficient to check the census figures or figures gathered by untrained village officials with those gathered by trained experts in a certain number of villages. Where high and low endemicity are found in neighbouring villages or areas a close comparison of the respective conditions will be very illuminating.

#### SUMMARY

(1) The chief direct causes of high endemicity of leprosy in India are dietetic, the poor diet available being supplemented by decomposing fish, meat, rice, etc.

(2) Caste and race have a certain bearing due to habits regarding diet and to certain aboriginal tribes being driven into the less fertile tracts or into serfdom or debt. Venereal diseases, which predispose to leprosy, are specially common among certain low castes.

(3) The poor soil of certain tracts, its porosity and the slope of the land are of importance, as the crops are correspondingly poor or precarious and dietetic errors result.

(4) While a very low humidity makes for a low incidence of leprosy as shown by Sir Leonard Rogers, this survey has not shown a close correlation between humidity and leprosy.

(5) Excessive rainfall by washing away and impoverishing the soil, by leading to mosquito-breeding and malaria and in other ways may have an indirect effect.

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# TUMOUR-LIKE GROWTHS CAUSED BY INTRAPERITONEAL INOCULATION OF *B. LEPRÆ MURIUM*

BY

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## INTRODUCTION

It may be asked why rat leprosy, a disease which with one or two exceptions has never been known to infect human beings, should be studied. The reasons are as follows —

- 1 The very close relationship to human leprosy, a relationship almost similar to that existing between human, bovine and avian tuberculosis
- 2 The fact that human leprosy cannot be inoculated into experimental animals, whereas much light may be shed on the human infection, as for example, the mode of entrance of the organisms into the body, its pathology, means of effective treatment, etc., by studying this closely related disease
- 3 The disease in rats is relatively rapid and almost 100 per cent of infections can be induced by inoculation
- 4 After finding the most favourable means of transmitting rat leprosy to rats it may be possible to inoculate human leprosy into the same animal

Various experiments are being tried along the lines mentioned above and the following paper is the first which is ready for publication —

Leprosy-infected rats were obtained through the kindness of Col J. Morison, F.R.S., Acting Director of the Haffkine's Institute, Bombay. From these, three



white and three wild decumanus rats were inoculated subcutaneously on 8th October, 1925. The three white rats developed the disease, and from one of these eight rats were inoculated subcutaneously and ten intra-abdominally on the 4th of March, 1926. All the rats which survived developed definite signs of rat leprosy within four to five months, but it was noticed that those inoculated intra-abdominally showed much grosser lesions than those inoculated subcutaneously. As the object of this paper is to describe a definite type of lesion, we shall only mention other pathological changes in so far as they bear on this subject.

Five of the intra-abdominally inoculated rats showed intra-abdominal spherical growths, viz —

(1) Rat A 29, inoculated intra-abdominally on 4th March, 1926, died on 8th August, 1926. There was a swelling the size of a small walnut in the right iliac fossa and another in the left iliac fossa.

(2) Rat A 27, inoculated intra-abdominally on 4th March, 1926, autopsied 14th July, 1926. Two growths were present, one under the liver on the right side and one in contact with the bladder in the left iliac fossa. Both of these growths showed central caseation and liquefaction. That on the left was adherent to the anterior abdominal wall, that on the right to the adjacent organs to a certain extent. A guinea-pig inoculated with some of the caseous matter showed no signs of tuberculosis.

(3) Rat A 28, inoculated intra-abdominally on 4th March, 1926, autopsied 22nd July, 1926. Three growths were present in the abdomen weighing 20.35 grms, 16.25 grms and 11.1 grms, respectively, these tumours were situated (a) underneath right lobe of the liver, (b) in front of the spleen—pushing forward the splenic flexure of the colon, (c) just above the symphysis pubis.

(4) Rat A 30, inoculated intra-abdominally on 4th March, 1926, autopsied 12th August, 1926. There were three intra-abdominal growths whose respective sizes and positions are best appreciated by reference to Plate I.

'a' was a large double growth situated apparently between the folds of the gastro-hepatic omentum, 'b' was the largest of the three and was situated in the right iliac fossa and attached to the epiploon, while 'c' was situated behind the hepatic flexure of the colon which was attached to, and pushed forward by, the neoplasm. There was also considerable splenic enlargement (Plate I 'd').

(5) Rat A 44, inoculated intra-abdominally on 4th March, 1926, autopsied 19th August, 1926. There was one large swelling the size of a pigeon's egg in the omentum, while the remainder of this structure was nodulated, the nodules being of all sizes up to a pea. The spleen was slightly enlarged and congested and was adherent to the omentum.

In contrast to the above animals in which definite signs of tumour-like formations were present, the post-mortem findings in two other rats are given namely —

(a) Rat A 49, inoculated intra-abdominally on 4th March, 1926, autopsied 9th August, 1926. The omentum was much hypertrophied, weighing 2.75 grms, its length was 10 cms. On palpating it, there was a sensation of nodulation or of coarse granulation (see Plate II, fig 2b).

(b) Rat A 26, inoculated intra-abdominally on 4th March, 1926, autopsied 7th August, 1926. There were no intra-abdominal swellings similar to those in the first five rats but both liver and spleen were much enlarged, especially the latter organ. The liver presented a mottled appearance and the spleen was covered with small, whitish nodules. The latter organ was 75 mm long by 17 mm broad and weighed 6.08 grms (see Plate II, fig 2b).

*Summary of post-mortem findings*—Out of the seven rats described above, five showed definite spherical growths, one to three in number and of various



RAT LEPROSY  
Intra Abdominal Tumour



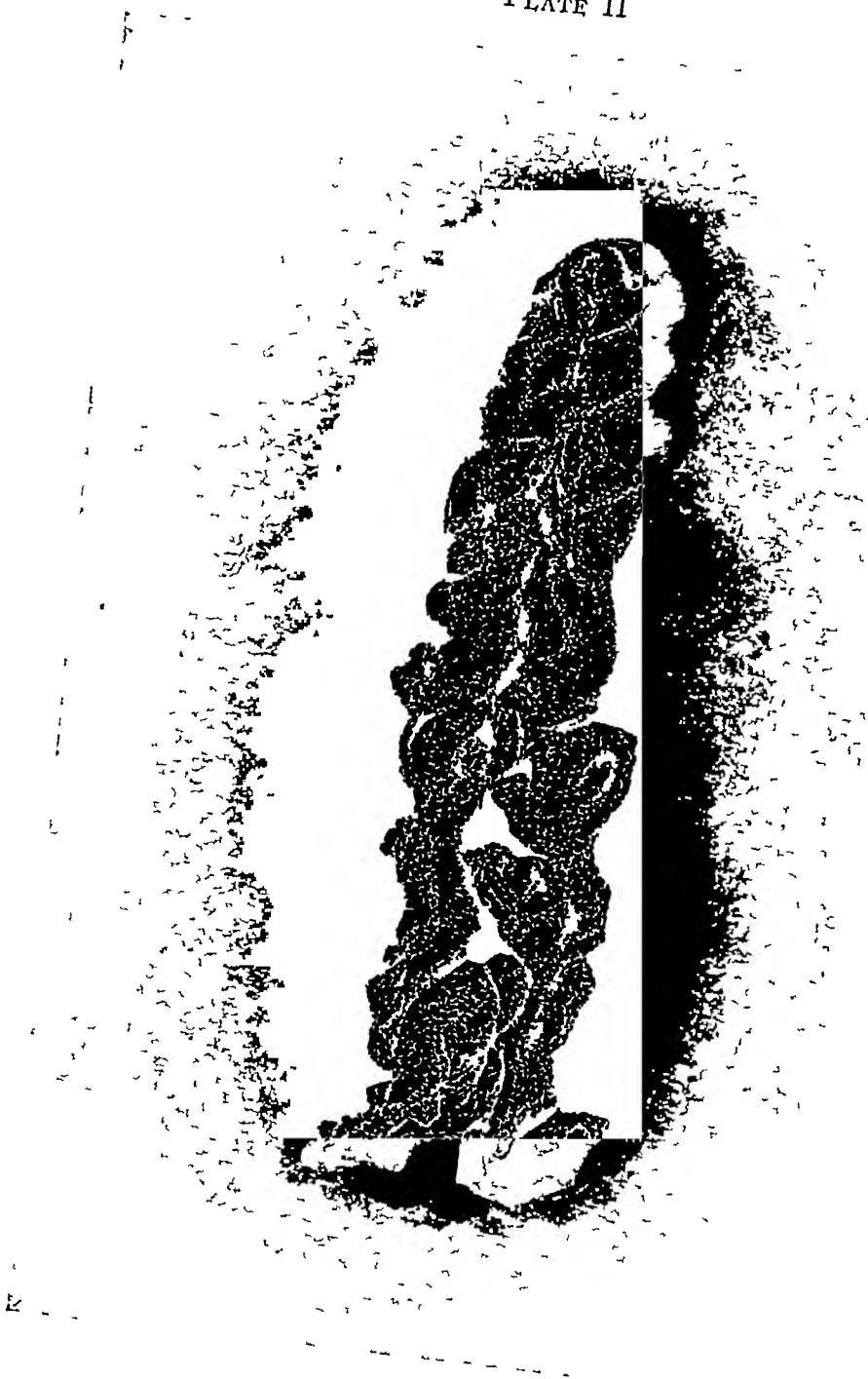


Fig 2b





# PLATE III

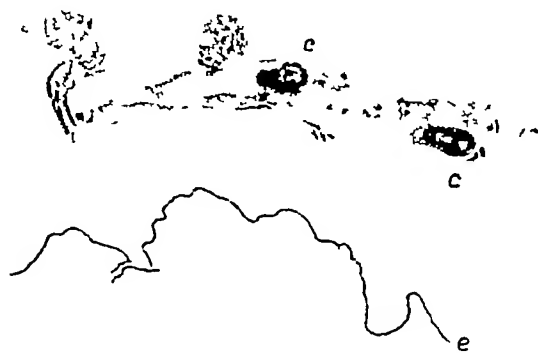
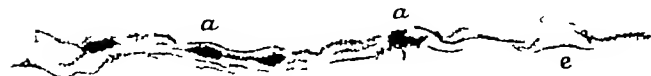
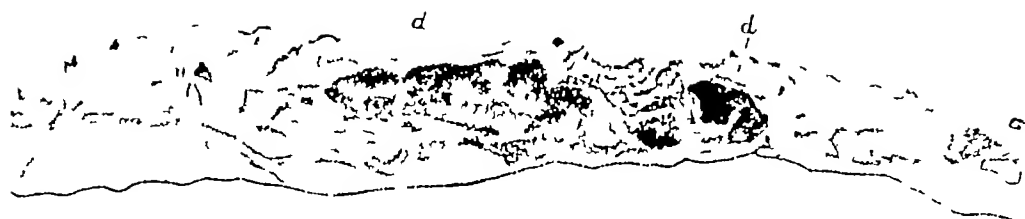




PLATE IV







sizes In Rat A 49 there was a more diffuse leprotic condition of the omentum without a tumour-like formation, while in Rat A 26 the omentum was not markedly enlarged, instead of this, the liver and spleen were much more intensely affected than in those rats in which there were signs of omental disease The feature common to all the spherical growths on section was the presence of an organised layer externally, lined by a thick caseous layer with liquefaction in the centre

*Microscopic examination*—1 There was an external layer 2 to 5  $\mu$  in thickness containing large round and spindle-shaped 'lepra' cells These cells were full of acid-fast bacilli which were so numerous as to obscure cellular details; the nuclei of the cells, however, appeared to be pushed to one side

2 Inside this was a highly vascular layer composed of spindle-shaped cells, fibrous tissue and small lymphocytes Many of these cells also contained acid-fast bacilli but to nothing like the extent obtaining in layer (1) (*vide supra*)

3 Still deeper was a layer composed of longitudinal fibrous strands showing very little cell structure

4 In the innermost part of the growth the fibrous structure became still less distinct and merged into a caseous, amorphous zone This last appeared in its deepest part to be undergoing liquefaction

*Discussion on the possible origin of these growths*—The occurrence of these spherical growths in the omentum suggested their possible origin from the peritoneum (*see* Plate I) Referring to this figure it will be noted that growth 'a' was lying between the layers of the gastro-hepatic omentum, splitting the layers of peritoneum at the point of junction with the stomach to which it was firmly bound down in front and behind along the lesser curvature

An endeavour was made to ascertain the origin of these growths by examining the peritoneal covering of various organs, and Plate III shows the different stages of infection of the peritoneal covering of the kidney with rat leprosy Cells marked 'a' and 'b' show the flattened endothelium in profile Many of these cells contain large number of bacilli 'c' shows the same cells somewhat enlarged, while 'd' shows large masses of 'lepra' cells It will be noticed that the peritoneum at this point is greatly thickened due to proliferation of endothelial cells, obviously with a view to phagocytosing the lepra bacilli 'e' is the outline of the kidney from which the peritoneum has been separated in places during the process of section-cutting

A nodule occurred on the corner of the spleen due to a process similar to that shown in Plate III 'd' but carried much further It was composed of innumerable 'lepra' cells whose contents stained darkly at the margins but showed signs of degeneration in the centre There were evidences of the process breaking through the capsule of the spleen at places, but the growth was almost entirely extracapsular Infection occurred at the centre of a Malpighian corpuscle probably by metastatic blood spread The supra-splenic tumour is therefore simply a continuation of the same process as formed the thickening of the renal peritoneum

We pass now to Plate IV, which represents a section through growth 'c' of Rat A 30 (*see* Plate I) To reconstruct the mode of origin of the growth it will be necessary to consider the findings in some of the other rats In the omentum

of Rat A 49 (*see* Plate II, fig 2*b*) we see a process similar to that which has taken place in the renal peritoneum and in the small supra-splenic tumour, but on a much larger scale, namely, thickening of the peritoneum due to stimulation of its endothelial cells by lepra bacilli, this induces mitotic division followed by phagocytosis of bacilli

Here, however, the process has gone much further. In Plate I we have a further stage in the series in which, instead of more or less general omental thickening, we have exaggerated nodule formation at certain points, these nodules growing apace till they become large tumour-like growths. The tendency to central autolysis is shown in the supra-splenic nodule. This degenerative change is still more marked in the intra-omental spherical growths where fibrosis, caseation and liquefaction take place one after the other at the centre of the tumour, and where also proliferation of the peritoneal endothelium, followed by phagocytosis of bacilli, tends to add to the size of the tumour.

Plate V shows the tendency towards autolysis and degeneration at the centre of growing nodules in the liver. We see lobules of the liver infected from the portal vein with proliferation of Kupffer cells and phagocytosis of bacilli which displaces and destroys through pressure the true hepatic cells 'a'. The typical lysis of 'lepra' cells at the centre while the process tends to spread radially, is also well shown.

Marchoux, 'Annales de l'Institut Pasteur,' Vol 37, 1923, p 342, referring to both human and rat leprosy, states that the 'leprous tubercle' has no tendency towards suppuration, it invades and causes compression and trophic troubles and disappearance of differentiated tissues ('le tubercule lepreux n' a pas de tendance a la suppuration il est envahissant et provoque par compression et troubles trophiques, la disparition des tissus différenciés')

Besides examining the method of growth of these granulomatous swellings there are some further points to be noted —

- (1) The almost invariable intracellular position of lepra bacilli
- (2) The way in which they are confined to certain cells, this is especially noticeable in the liver where the hepatic cells themselves are not invaded by bacilli, but are destroyed by pressure of the lepra cells
- (3) The protective power of the omentum. Spleen and liver are little affected in those rats in which the omentum is grossly involved and, conversely, they are greatly involved in rats in which the peritoneum has failed to take on this rôle, as in Rat A 26
- (4) A point still to be determined is to what extent spleen and liver are attacked directly from their peritoneal coverings and to what extent they are affected by metastatic blood spread
- (5) As to the nature of the cells which are stimulated to multiplication and phagocytosis by lepra bacilli, this matter is still under review. There seems much to be said for the view of Oliver (*Journal of Experimental Medicine*, 43 233—239, February, 1926) that the cells of the reticulo-endothelial system are specially involved
- (6) The rôle of the pathogenic and saprophytic organisms, which are almost inevitably inoculated along with the leprous material, and which are usually

PLATE V





discoverable in the substances of the spherical growths, in preparing a nidus for the formation of these growths is a point which has still to be determined.

Here we have a definite, rapidly-growing, granulomatous growth with a very clear pathology, this may point the way towards the illumination of the pathology of other growths more obscure in etiology.

#### SUMMARY

(1) The reasons which induced us to take up the study of rat leprosy are as follows —

- (a) The very close association between this disease and human leprosy
- (b) Human leprosy cannot be conveyed to lower animals, whereas almost 100 per cent of successes can be assured by inoculating rats with *B. lepræ murum* provided the appropriate technique is used
- (c) The course of the disease in the rat is a relatively rapid one

(2) As will be observed from the title, we discovered that structures resembling tumour growths occurred in the abdominal cavity following on the intra-abdominal inoculation of rat leprosy. Such growths, there is reason to believe, are granulomatous and are due to multiplication of the peritoneal endothelial cells and phagocytosis of bacilli together with multiplication of bacilli inside cells. We have also remarked on the fibrosis, caseation and liquefaction which occur in the tumours.

(3) We think that a description of the mode of origin of these growths may help to shed some light on the pathology of human leprosy and also, possibly, on the origin of other new growths more obscure in etiology.



# NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

## Part XIX

### THE VALUE OF THE FEMALE GENITALIA IN THE IDENTIFICATION OF SPECIES

BY

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THE identification of the female specimens of *Phlebotomus* has always been the greatest difficulty encountered by workers with this genus. It is only the females of this genus which suck blood and are, therefore, potential carriers of disease, so it is of the greatest practical importance that a reliable method of identification of specimens of this sex should be available.

Recently a preliminary study of the female genitalia of *Phlebotomus* was undertaken (Sinton, 1925*b*), with the object of determining whether these organs revealed any points of diagnostic importance equal in value to those shown by the homologous parts in the male. The conclusions arrived at in this work were that 'in our present state of knowledge of the female hypopygium of *Phlebotomus*, it was impossible to compile any diagnostic table based on the structure of the organ until more specimens are examined, the range of variation determined, and the degree of constancy of the different features known' (Sinton, 1925*b*, p 106).

Since that time the writer has examined the female genitalia, both internal and external, of a large number of 'sandflies' from India and has found that in several species the morphology of certain parts of these structures show features of primary diagnostic value.

Newstead (1911), in his diagnostic table of the Maltese 'sandflies' has divided the species found by him into two groups (a) those in which the hairs on the dorsal surface of the abdominal segments are erect, and (b) those in which these hairs are recumbent. A similar grouping was used in a previous paper of this series (Sinton, 1925*a*), in compiling a provisional diagnostic table of the males of the Indian *Phlebotomus*.



It sometimes happens, however, that the hairs on the dorsal surface of the abdomen may be rubbed off, and so it is difficult to determine into which group of Newstead's division a specimen should be placed. In specimens which have been treated with caustic potash solution, stained and mounted (*vide* Sinton, 1925a), this difficulty can usually be overcome by an examination of the dorsal surface of the abdominal segments at their distal margins. The very distinct, large, circular scars left at the points of attachment of erect hairs (Plate VI, fig 1, E) are markedly different from the fainter, smaller and more triangular scars left by the attachments of recumbent hairs (Plate VI, fig 1, R).

This grouping is almost identical with that arrived at by a study of the female genitalia, where it was found that in certain species the outline of the chitinisation of the spermathecae was smooth, while in other species the outline had a markedly crenulated appearance. The group with the 'crenulated' spermathecae is identical with the 'erect-haired' group of Newstead and those with the 'smooth spermathecae' with the 'recumbent-haired' group, except in the case of *P. squamipleuris* which seems to be intermediate between these two groups. Certain other less marked differences have been found between these groups. These will be described later.

This method of dividing the genus *Phlebotomus*, according to the morphology of the spermathecae, has been found of the greatest practical value in the identification of the members of this genus. *P. similis* var. *hospiti* was originally described as belonging to the 'recumbent-haired' group (Sinton, 1924b) \*. The male hypopygium of this species resembles very closely that found in *P. minutus* and its varieties, a 'recumbent-haired' species, but an examination of its spermathecae (Plate VI, fig 8), at once, shows that it really belongs to the 'erect-haired' group. A careful examination of the types and co-types of *P. similis* var. *hospiti* revealed the presence of the scars of some erect hairs on the dorsum of the abdomen and this has since been confirmed by the examination of other specimens caught at Kasauli, Punjab.

Similarly in 1923 a number of *Phlebotomus* were collected at Lahore by the writer and isolated from the other specimens of the *minutus* group, which they closely resemble, because they showed a few erect hairs on the dorsum of the abdomen. It was, at first, thought that the condition was caused by the specimens having been rubbed at the time of capture, but an examination of their spermathecae has shown that these insects belong to the 'erect-haired' group, although the male genitalia are of the *minutus* type. This is a new species for which the name *Phlebotomus christophersi* is proposed. It will be described in detail in a later article.

The females of *P. similis* var. *hospiti* and of *P. christophersi* have both recumbent and erect hairs on the dorsal surfaces of their abdomens, but the erect hairs are fewer than those seen in the other members of the 'erect-haired' group. In the males the erect hairs are usually much scantier and may not be

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\* Since writing the above I have had the opportunity of examining a number of *P. similis* from Africa. The African species is a 'recumbent-haired' one and quite distinct from the Indian *P. similis* var. *hospiti* which should, therefore, be raised to specific rank.

detected in unmounted specimens, but in specimens, which have been stained and mounted, the scars of, at least, a few erect hairs are distinctly visible on the dorsum of the 2nd abdominal segment

*P. squamipennis* on careful examination sometimes shows a few erect hairs, mainly on the dorso-lateral surfaces of the abdomen, but the spermathecae have not the distinctly crenulated outline seen in the other members of the 'erect-haired' group, although they show transverse markings (Plate VI, fig 11) In certain other features *P. squamipennis* resembles the 'recumbent-haired' group rather than the other one, so it would appear rather to occupy an intermediate position

#### THE SPERMATHECÆ

Grassi (1907) described and figures these structures in *P. papatasu* Newstead (1911) also describes them and states that they consist 'of a single-walled, sub-spherical sack, and are relatively very large, at their junction with the duct they are strongly chitinised, and consist of usually ten transverse and convex ridges, which are so constricted at the margin as to present, in optical section, a distinct and well-marked crenulation' (p 164) This description apparently applies to *P. papatasu* No fresh description of the morphology of these structures seems to have been given until Adler and Theodor (1926) figured them in *P. minutus*, in *P. minutus* var *africanus* and in *Phlebotomus* sp from Palestine The latter workers called attention to very slight differences in the morphology of the spermathecae of the species described by them These species belong to the 'recumbent-haired' group in the spermathecae of which the writer has also been unable to find features of any great diagnostic value, but in the 'erect-haired' group some of the species have a morphology, which seems to be specific In specimens treated with caustic potash solution and stained, it is the chitinisation at the base which is most evident and it is this portion which is described in this article

*The 'Erect-haired' Group* In this group the crenulated chitinisation described by Newstead at the base of the spermathecae is capable of extension and contraction like a concertina In the retracted state the transverse ridges appear almost linear and it is often difficult to determine how many there are (Plate VI, figs 2, 5, 6, 9 and 14) The chitinisation is usually much denser than in the 'recumbent-haired' group

At the end of the chitinisation furthest from the oviduct is a structure from which a number of hair-like structures project (Plate VI, figs 3, 7, 8, 11, 12, 13 and 17, H) This is here called the 'head' for convenience of description

The following Indian species belonging to this group have been examined -- *P. papatasu*, *P. major*, *P. argentipes*, *P. simillimus* var *hospitu* and *P. christophersi*.

(a) *P. papatasu* When the spermatheca is expanded, it is somewhat sausage-shaped with about 10 transverse ridges (Plate VI, fig 3) The 'head' is as wide as the body of the structure and is of a characteristic shape (Plate VI, figs 2 and 3, H) When the structure is contracted, it is usually difficult to determine the number of ridges present but the characteristic 'head' can easily be made out (Plate VI, fig 2)

(b) *P. major* The body is sausage-shaped (Plate VI, fig 7) and rather resembles that of *P. papatasi*, but the head is set on a characteristic long neck, which is apparently not retractile (Plate VI, fig 6, N) The head is little wider than the diameter of the neck

(c) *P. argentipes* The spermatheca is a characteristic carrot-shaped structure (Plate VI, fig 4) The crenulations are about 15 in number and gradually diminish in size until they become continuous with the oviduct The head is small and has no long neck The shape of the body is somewhat obscured in contracted specimens (Plate VI, fig 5)

(d) *P. simillimus* var *hospiti* Here the spermatheca is very like that seen in *P. papatasi* but the head is much narrower than the body and has a different morphology from that species (Plate VI, figs 8 and 9) The crenulations are about 10 in number \*

(e) *P. christophersi* Here the spermatheca resembles that of the last specimen but it has fewer crenulations (Plate VI, figs 13 and 14) \*

These are all the members of the 'erect-haired' group known to occur in India except *P. sergenti*, of which unfortunately no undoubted specimen of the female was available It is seen, however, that, amongst the species examined, *P. papatasi*, *P. major* and *P. argentipes*, at least, have a very distinctive morphology The morphology has been found of great use in the identification of the females of these species

(f) *P. squamipleuris*, on account of its few erect abdominal hairs, should be placed in this group Its spermatheca has not, however, the distinct crenulations seen in the other members of this group and does not seem to be contractile The structure is turnip-shaped and is usually a conspicuous object in stained specimens Although not crenulated it has a series of transverse rows of small projections on its outer surface (Plate VI, fig 11) The hairs which project from the 'head' are very numerous and stain deeply, giving this structure the appearance of a shaving brush The morphology of the spermatheca seems to be diagnostic of the females of this species, for although a very large number of Indian 'sandflies' have been examined by the writer, in no other species has the same morphology been found

*The 'Recumbent-haired' Group* In this group the outline of the spermatheca is smooth The chitinisation is much less dense than that seen in the 'erect-haired' group For this reason it is often very difficult or impossible to ascertain accurately the details of these structures in stained and mounted preparations and for the same reason they are often distorted Adler and Theodor (1926) have figured the spermathecae in three members of this group and it can be seen that the differences depicted by them are too slight to be of much diagnostic value Similarly the writer has found that the difficulty in examining these structures, the frequency of distortion and the slight variations in morphology have, up to the present, made these structures of very little use in the

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\* The palpal formula of *P. simillimus* var *hospiti* is 1, 2, 3, 4, 5, while in *P. christophersi* it is 1, 2, 4, 3, 5

specific diagnosis of the Indian species of *Phlebotomus* which belong to this group

(a) *P. malabaricus* (?) \* The spermatheca is cigar-shaped with a halo-like head from which a few hairs project (Plate VI, fig 10)

(b) *P. minutus* var *antennatus* Here the structure resembles the head of a pipe The 'head' is sunken into the opening of the pipe and has only a few hairs (Plate VI, fig 12)

(c) *P. minutus* var *montanus* (Plate VI, fig 15) and *P. minutus* var *niger* (Plate VI, fig 18) have both spermathecae which are larger and more egg-shaped

The *minutus* group in India is a very complicated one, for different tracts seem to have their own special type of this group These different types have not as yet been separated out into different varieties or species, but have all been grouped under the common heading of '*P. minutus* var' The spermatheca of a variety from Pachmarhi, Central Provinces, is figured (Plate VI, fig 17) and also one from Assam (Plate VI, fig 16) These two spermathecae are seen to have distinctly different appearances The palpal formula of the former variety is 1, 2 (3, 4), 5, and of the latter 1, 2, 3, 4, 5

#### THE FURCA

The thickened chitination of the handle of the furca (Sinton, 1925b) in the 'erect-haired' group either ends or divides into two portions at some distance before it reaches the bottom of the hollow of the fork (Plate VII, figs 19 to 21 and 24 to 25), while in the 'recumbent-haired' group it is continued backwards as a solid bar almost to the bottom of the hollow (Plate VII, figs 22 and 26 to 28) The furca of *P. squamipleuris* resembles those of the latter group (Plate VII, fig 23)

(a) *P. papatasi* (Plate VII, fig 19) The limbs of the furca are broad and flat An internal and an external projection can usually be seen about their middle (Plate VII, fig 19, I and E) The base of the furca is deeply fringed and is wider than the fork

(b) *P. major* (Plate VII, fig 24) The limbs of the furca are also broad and flat in this species but have not the projections seen in *P. papatasi* The base has a few serrations at the distal margin of the outer angles The whole structure is comparatively longer and narrower than in the other members of this group

(c) *P. argentipes* (Plate VII, fig 20) The limbs are narrow and the distal portions have the appearance of a ribbon turned on edge The space enclosed by the limbs is much more nearly circular than in the previous two species The base has a number of shallow serrations The handle is stumper than in the other two species and the chitination divides earlier

(d) *P. simillimus* var *hospiti* The handle is narrow and its chitination shows only a slight tendency to forking The base has few or no serrations (Plate VII, fig 21)

(e) *P. christophersi* (Plate VII, fig 25) The furca in this species resembles that in the one last described but the handle is narrower and longer, and the base broader

\* This is the specimen described in a previous paper (Sinton, 1927) as probably the female of *P. malabaricus*

The figures give a better idea of the morphology of these structures than any verbal description. It will be seen that the morphology of the furca in *P. papatasu*, *P. major* and *P. argentipes* shows distinct features which may be of value in diagnosis, but that in *P. simillimus* var. *hospitu* and in *P. christophersi* they do not show any very characteristic points.

The 'recumbent-haired' group. The furca was examined in *P. malabaricus* (?), *P. minutus* var. *antennatus*, *P. minutus* var. *niger*, *P. minutus* var. *montanus* and *P. minutus* var. , but as will be seen from the figures the differences between the structures in these species was so slight as to be of little value in diagnosis (Plate VII, figs 22 and 26 to 28). The furca in *P. squamipleuris* resembles that found in the 'recumbent-haired' group (Plate VII, fig 23).

#### THE POST-GENITAL PLATE AND RIDGE

If the shape of the post-genital plate in the 'erect-haired' group be compared with that of the 'recumbent-haired' group, it is seen that in the former group its shape is squarer than in the latter group, in which it is by comparison narrower and more elongated transversely. The difference is, however, not very marked. The plate in *P. squamipleuris* resembles that in the 'recumbent-haired' group (Plate VII, fig 36).

The number of spines on the post-genital ridge in the different species of Indian *Phlebotomus* is in the majority of specimens constant for that species, but variations occur. These variations have so far prevented this feature from being of much use in the identification of species. Whether the specimens, which apparently have an abnormal number of spines, are distinct races of the type species has yet to be determined.

(a) *P. papatasu*. The post-genital plate (Plate VII, fig 31) in this species is usually composed of two portions, a feature not yet found in any of the other species examined. There are usually no large spines on the ridge, but very rarely two spines, like those seen in *P. major* have been seen in specimens which are apparently *P. papatasu*.

(b) *P. major* (Plate VII, fig 30) has two widely separated spines on the post-genital ridge, but more rarely three spines have been found.

(c) *P. argentipes* (Plate VII, fig 32) has two spines like *P. major*, but these are less widely separated.

(d) *P. simillimus* var. *hospitu* and *P. christophersi* (Plate VII, fig 35) have four spines on the post-genital ridge. These are closely grouped.

(e) *P. squamipleuris* (Plate VII, fig 36) and *P. minutus* var. *montanus* (Plate VII, fig 33) have each two spines close together.

(f) Three is the usual number of spines found in *P. minutus* var. *antennatus* (Plate VII, fig 37), while four is the number seen in *P. perturbans*, *P. minutus* var. *niger* (Plate VII, fig 29) and *P. malabaricus* (?) (Plate VII, fig 34). In some specimens of *P. minutus* var. five spines are present.

#### THE EIGHTH ABDOMINAL STERNITE

In *P. squamipleuris* and all the members of the 'recumbent-haired' group examined, the junction between the two halves of the eighth sternite is deep and

straight (Plate VII, fig 38) Amongst the members of the 'erect-haired' group the junction is narrow and peaked (Plate VII, fig 39), except in *P christophersi* which seems to be variable

### CONCLUSIONS

1 The morphology of the spermathecae of the Indian species of *Phlebotomus* examined allows of the division of the members of this genus into two groups—(a) those with 'crenulated' spermathecae, and (b) those with 'smooth' spermathecae These groups correspond very closely with the 'erect-haired' and the 'recumbent-haired' groups described by Newstead (1911)

2 The morphology of the spermathecal chitinisations show characters in several of the species of the 'erect-haired' group which are apparently specific In the 'recumbent-haired' group these structures have been found of little value in specific diagnosis

3 The morphology of the furca may also be of diagnostic aid in the 'erect-haired' group

4 The number of spines on the post-genital ridge is of some help in diagnosis

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## DESCRIPTION OF PLATE VI

The drawings were made with the camera-lucida from stained and mounted specimens. The scale of measurement of all the figures, except Plate VI, fig 1, is given at the foot of the plate.

- Fig 1 Dorso-lateral portion of the posterior margin of the 5th abdominal segment of *P christophersi* (♀), showing the types of scars left by erect (E) and recumbent hairs (H)
- „ 2 Spermatheca of *P papatasu* in contracted condition H 'head' of spermatheca
- „ 3 Spermatheca of *P papatasu* in extended condition
- „ 4 Spermatheca of *P argentipes* in extended state
- „ 5 Spermatheca of *P argentipes* in contracted state
- „ 6 Spermatheca of *P major* in contracted state
- „ 7 Spermatheca of *P major* in extended state N is 'neck' and H the head
- „ 8 Spermatheca of *P similimus* var *hospiti* in extended state
- „ 9 Spermatheca of *P similimus* var *hospiti* in contracted state
- „ 10 Spermatheca of *P malabaricus* (?)
- „ 11 Spermatheca of *P squamipleuris*
- „ 12 Spermatheca of *P minutus* var *antennatus*
- „ 13 Spermatheca of *P christophersi* in extended state
- „ 14 Spermatheca of *P christophersi* in contracted state
- „ 15 Spermatheca of *P minutus* var *montanus*
- „ 16 Spermatheca of *P minutus* var from Assam
- „ 17 Spermatheca of *P minutus* var from Pachmarhi
- „ 18 Spermatheca of *P minutus* var *inger*

# PLATE VI.

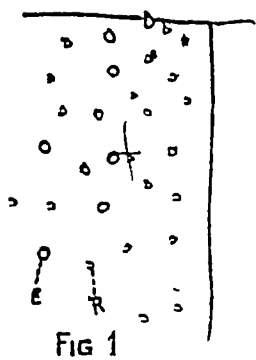


FIG 1

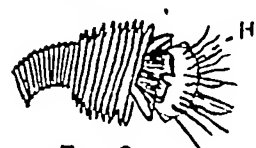


FIG 2



FIG 10

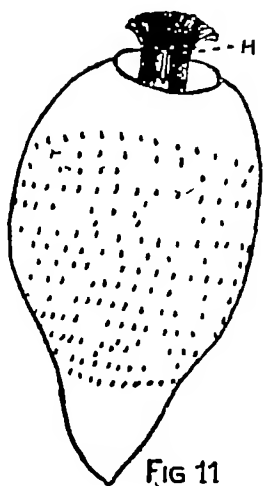


FIG 11

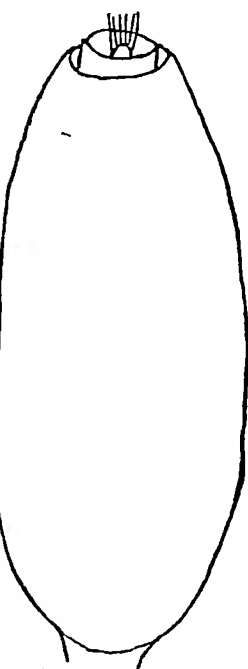


FIG.15

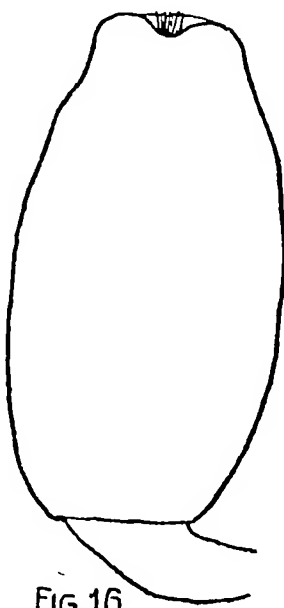


FIG 16

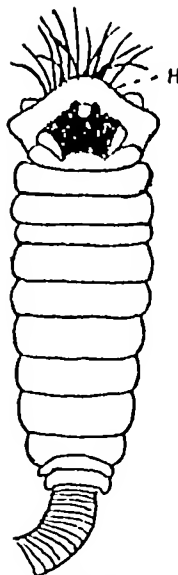


FIG 3

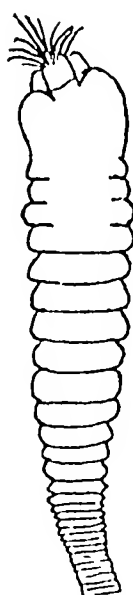


FIG 4

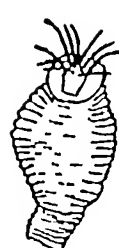


FIG 5



FIG 6

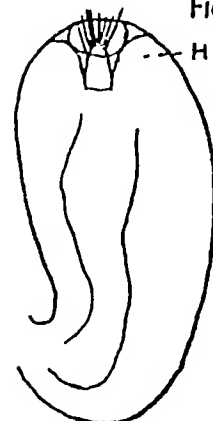


FIG 12

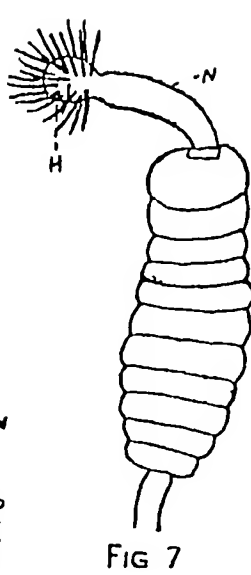


FIG 7

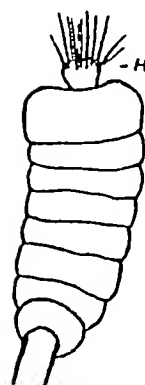


FIG 13

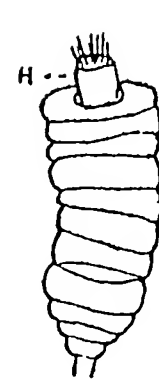


FIG.8



FIG 9

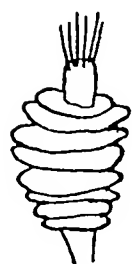


FIG 14

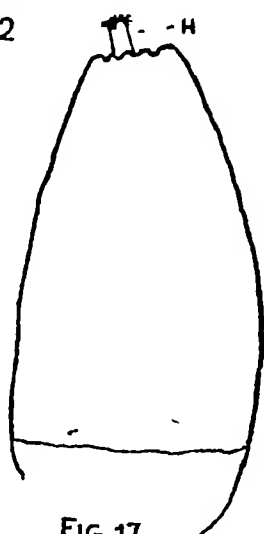


FIG 17

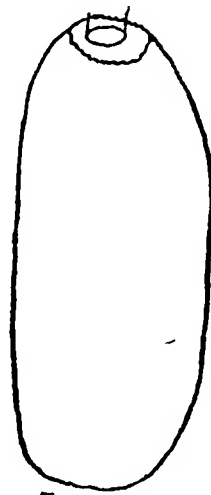
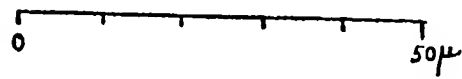


FIG 18





## DESCRIPTION OF PLATE VII

The drawings were made with the camera-lucida from stained and mounted specimens. The scale of measurement for all the figures is that shown between Plate VII, figs 19 and 20.

- Fig 19 Furca of *P papatasu*, showing internal (I) and external projections (E) on the limbs
- , 20 Furca of *P argentipes*
  - „ 21 Furca of *P similimus* var *hospitu*
  - „ 22 Furca of *P minutus* var *niger*
  - „ 23 Furca of *P squamipleuris*
  - „ 24 Furca of *P major*
  - „ 25 Furca of *P christophersi*
  - „ 26 Furca of *P malabaricus* (?)
  - „ 27 Furca of *P minutus* var *antennatus*
  - „ 28 Furca of *P minutus* var *montanus*
  - „ 29 Post-genital plate and ridge of *P minutus* var *niger*
  - „ 30 Post-genital plate and ridge of *P major*
  - „ 31 Post-genital plate and ridge of *P papatasu*
  - „ 32 Post-genital plate and ridge of *P argentipes*
  - „ 33 Post-genital plate and ridge of *P minutus* var *montanus*
  - „ 34 Post-genital plate and ridge of *P malabaricus* (?)
  - „ 35 Post-genital plate and ridge of *P christophersi*
  - „ 36 Post-genital plate and ridge of *P squamipleuris*
  - „ 37 Post-genital plate and ridge of *P minutus* var *antennatus*
  - „ 38 Eighth abdominal sternite of *P squamipleuris* (♀)
  - „ 39 Eighth abdominal sternite of *P papatasu* (♀)

PLATE VII.

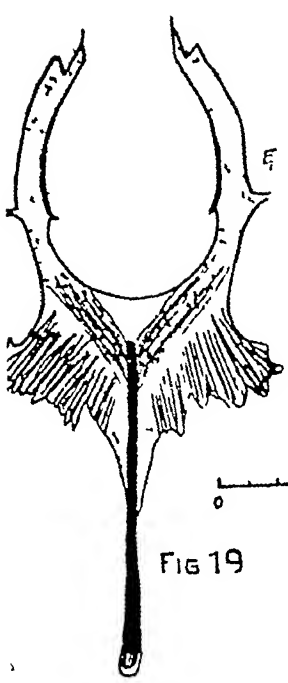


FIG 19

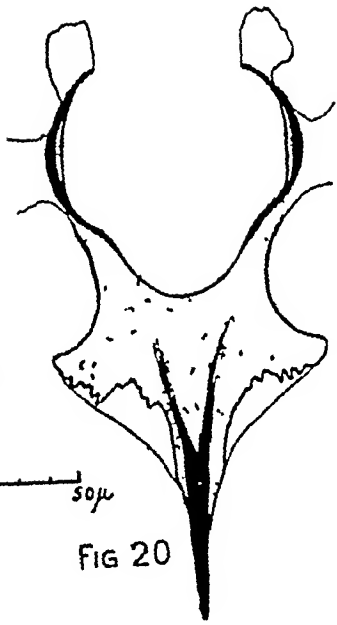


FIG 20

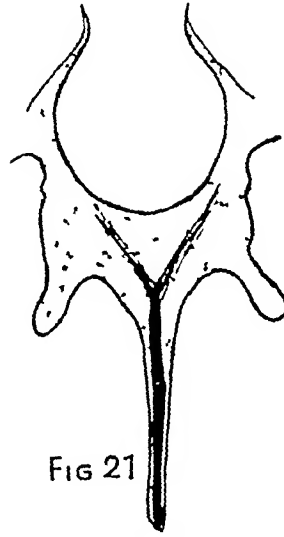


FIG 21

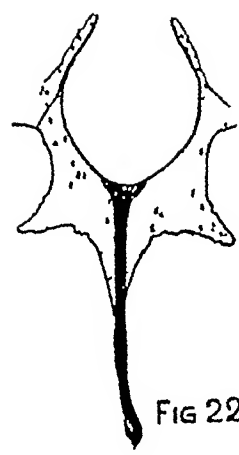


FIG 22

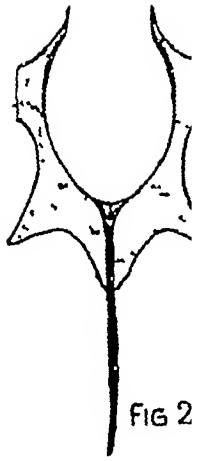


FIG 2

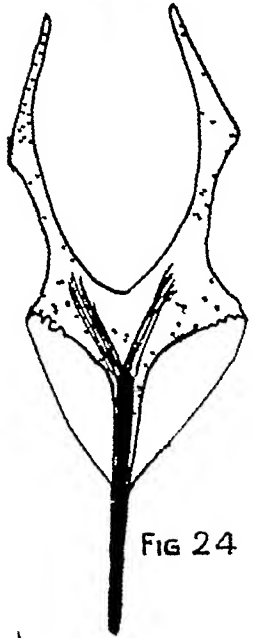


FIG 24

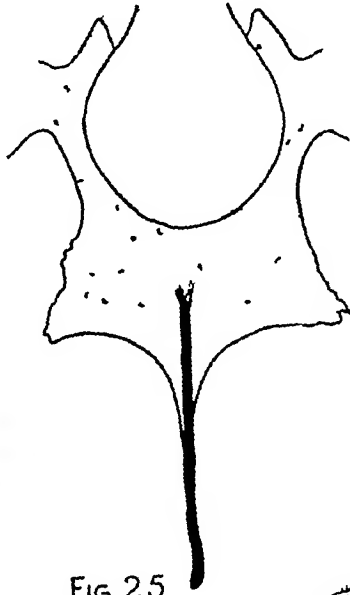


FIG 25

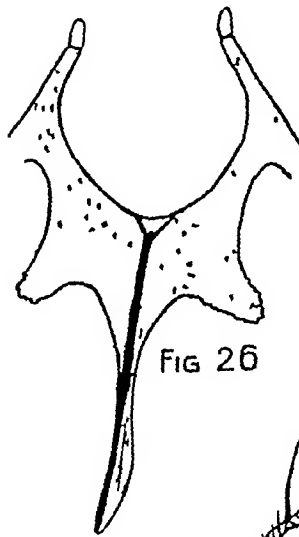


FIG 26

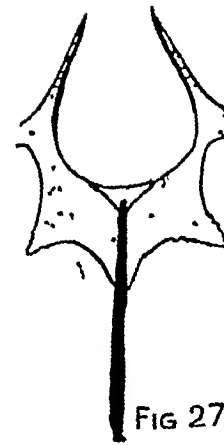


FIG 27

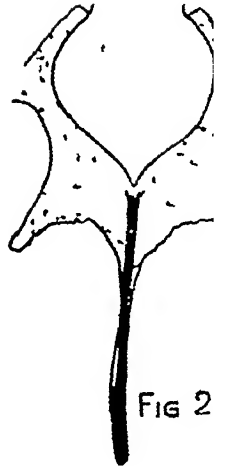


FIG 2

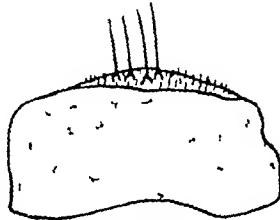


FIG 29

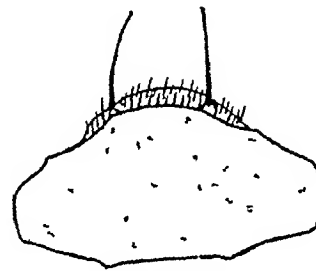


FIG 30

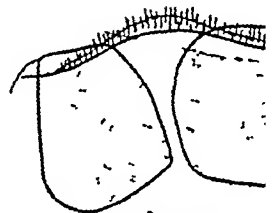


FIG 31

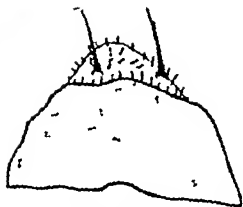


FIG 32

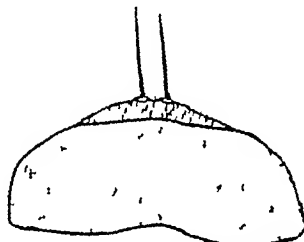


FIG 33

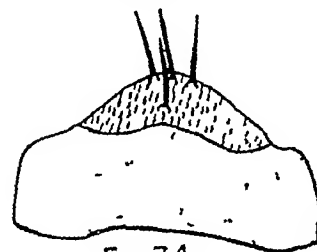


FIG 34

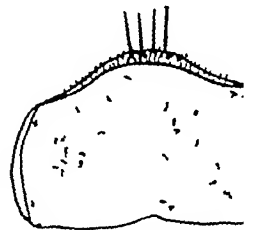


FIG 35

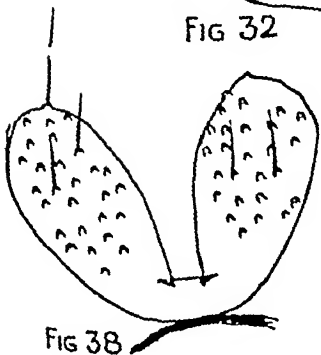


FIG 38

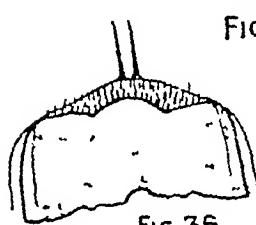


FIG 36

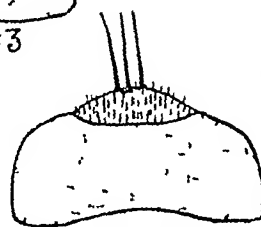


FIG 37

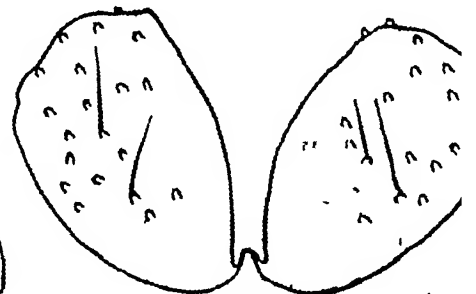


FIG. 39



# NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

## Part XX

### THE MORPHOLOGY OF THE BUCCAL CAVITY IN SOME SPECIES

BY

MAJOR J A SINTON, I M S

[Received for publication, February 7, 1927]

ADLER and Theodor (1926), in a study of the *minutus* group of the genus *Phlebotomus* in Palestine have drawn attention to the peculiar morphology of the buccal cavity and the pharynx of these insects. They think that 'possibly the characters of the pharynx and buccal cavity combined, or even if the pharynx alone, may prove to be of diagnostic value throughout the whole genus' (p 400).

These authors give the following description of the portion of the buccal cavity which they have found to be of diagnostic importance—'the posterior part of the floor of the buccal cavity of the *minutus* group bears teeth, and the arrangement of these teeth is probably of diagnostic value, for they are constantly present in males and females. In addition, the posterior dorsal part of the buccal cavity contains a pigmented area which is nearly always present in the female and is occasionally present in the male. The buccal cavity of *P. papatasi* and *P. perniciosus* contains no teeth and no pigmented area' (p 401).

It was pointed out in another paper of this series (Sinton, 1927b) that although the morphology of the spermathecae is of great value in distinguishing the members of the 'erect-haired' group of Indian *Phlebotomus*, yet it is not very useful in the 'recumbent-haired' one. It has now been found that the morphology of the posterior portion of the buccal cavity of those Indian species examined, affords considerable help in the diagnosis of the species belonging to the latter group, while it is of much less value in the former one.

The descriptions given in this paper were made from specimens treated with caustic potash solution and afterwards stained and mounted (*vide* Sinton, 1925). To get the most satisfactory view, the buccal portion was dissected out from the detached head while the specimens were in Canada balsam, before the cover-slip was applied. Adler and Theodor (1926), however, state that the characters

of this structure can be readily determined in fresh specimens mounted in saline. It is often very difficult to determine accurately the shape of the free ends of the teeth, whether they are flattened, rounded or pointed, so this character has not been used in diagnosis.

#### THE 'ERECT-HAIRED' GROUP

In the 'erect-haired' group the examination of the females of *P. papatasi*, *P. major* and *P. argentipes* and the male of *P. sergenti* failed to reveal any pigmented area similar to that seen in the 'recumbent-haired' group and, where teeth were present they were inconspicuous in comparison with those seen in the other species examined. In *P. simillimus* var. *hospiti* the development of the pigmented area and of the teeth is well-marked, while in *P. christophersi* they are poorly developed.

(a) *P. simillimus* var. *hospiti*. In both sexes the teeth are numerous and well-developed (Plate VIII, figs 1 and 2). The pigmented area in the male is large and oval, while in the female it is much larger and has in addition a short blunt tongue projecting forwards.

(b) *P. christophersi*. The pigmented area in both sexes is very small and rather carrot-shaped (Plate VIII, figs 4 and 5). The female has four or five large widely separated teeth (Plate VIII, fig 4), while in the male there are two or three large teeth and a number of much smaller ones (Plate VIII, fig 5). In the female there is a chitinous thickening joining the two lateral walls of the buccal cavity, just anterior to the position of the teeth (Plate VIII, fig 4, C).

(c) *P. squamipleuris*. The pigmented area in the female is shaped like the segment of an orange and from the middle of the straight or anterior edge it has a small pointed projection (Plate VIII, fig 7). In the male this area is much smaller and of an irregular oval shape (Plate VIII, fig 8). The female has a well developed row of teeth, while in the male they are much smaller and slightly separated from each other.

In both the male and the female the lateral chitinations of the buccal cavity show a large lateral projection on either side (Plate VIII, figs 7 and 8, P). Such a large projection has not been noted in any of the other species examined. In the female this projection has a number of serrations projecting posteriorly, but in the male it is rather more irregular in shape.

#### THE 'RECUMBENT-HAIRED' GROUP

In this group the teeth and the pigmented area were well-developed in all the species examined.

(a) *P. malabaricus* (?)\*. The pigmented area is large and of a triangular shape in the female (Plate VIII, fig 6). The teeth are large and well-developed.

(b) *P. montanus*. This species has a palpal formula of 1, 2, 3, 4, 5, the relative lengths of the segments being 2, 2, 5, 7, 8, 8, 10, 19, 2. The pigmented area of the buccal cavity in both sexes differs markedly from that seen in any of the

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\* These are the specimens described in a previous paper as probably the females of *P. malabaricus* (Sinton, 1927a).

other species of *Phlebotomus* examined (Plate VIII, figs 10 and 11) The teeth are also peculiar in both sexes There is a single row of teeth in the middle and about four rows at the margins These features combined with its other morphological peculiarities would seem sufficient to raise this insect from the varietal rank of *P. minutus* var *montanus* to specific rank as *P. montanus*

(c) The *minutus* group This group is a very confused one and it has been found that in India, with its great variations in physiography, different areas have their own special members of this group These insects in many instances show a marked variation in morphology, although the males have all the *minutus* type of genitalia They have, up to the present, mostly been grouped together as *P. minutus* var, but a more thorough study of these insects will probably reveal that several varieties or even species have been grouped together under this name This has already happened in the case of *P. montanus* and *P. christophersi* The group will require much more work before their classification is properly settled

Adler and Theodor (1926) have described the buccal cavity in two members of this group from Palestine, namely, *P. minutus* and *P. minutus* var *africanus*

(1) *P. minutus* var *antennatus* This variety closely resembles the type species, *P. minutus*, but differs in its very short 3rd antennal segment and its palpal formula—1, 2, 3, 4, 5 The buccal cavity closely resembles that figured by Adler and Theodor (1926) in *P. minutus* There is a well-marked row of teeth in both sexes and the pigmented area is oval in shape (Plate VIII, figs 12 and 13)

(2) *P. minutus* var *niger* This species has a palpal formula of 1, 2 (3, 4), 5 The pigmented area is rather mushroom-shaped with a depression on the top (Plate VIII, fig 9) The teeth are numerous There is a bar joining the two lateral walls of the cavity just anterior to the base of the teeth and this bar has an oval depression in its posterior free edge This type of buccal cavity rather resembles that depicted by Adler and Theodor (1926) in *P. minutus* var *africanus*, but in *africanus* the palpal formula is 1, 2, 3, 4, 5

(3) *P. minutus* var This is the variety seen in the North-eastern parts of India and in Rangoon, Burmah The buccal cavity resembles that seen in *P. minutus* var *niger*, except that the number of teeth are fewer and much larger (Plate VIII, fig 3)

The *niger* type of buccal cavity is that most commonly found in the *minutus* group in India Specimens showing the morphology figured by Adler and Theodor (1926) in *P. minutus* are much rarer, so it is possible that *P. babu* Annandale, 1910, may again be raised to specific or varietal rank

#### CONCLUSIONS

1 The morphology of the buccal cavity has been examined in a large number of specimens of Indian *Phlebotomus* and seems to be constant for the species examined

2 Amongst the species of *Phlebotomus* described in this paper, the morphology of the buccal cavity would appear to be of much diagnostic value, more especially in the 'recumbent-haired' group

#### DESCRIPTION OF PLATE VIII

The drawings were made with the camera-lucida from stained and mounted specimens. The scale of measurement is the same in all the figures (*vide* Plate VIII, fig 2). The posterior portion of the buccal cavities of the following species are depicted —

- Fig 1 *P similimus* var *hospitu* (♀).  
„ 2 *P similimus* var *hospitu* (♂).  
„ 3 *P minutus* var (♀) from Rangoon, Burmah  
„ 4 *P christophersi* (♀)  
„ 5 *P christophersi* (♂)  
„ 6 *P malabaricus* (?) (♀)  
„ 7 *P squamipleuris* (♀)  
„ 8 *P squamipleuris* (♂)  
„ 9 *P minutus* var *niger* (♀)  
„ 10 *P montanus* (♀)  
„ 11 *P montanus* (♂)  
„ 12 *P minutus* var *antennatus* (♀)  
„ 13 *P minutus* var *antennatus* (♂)

PLATE VIII.

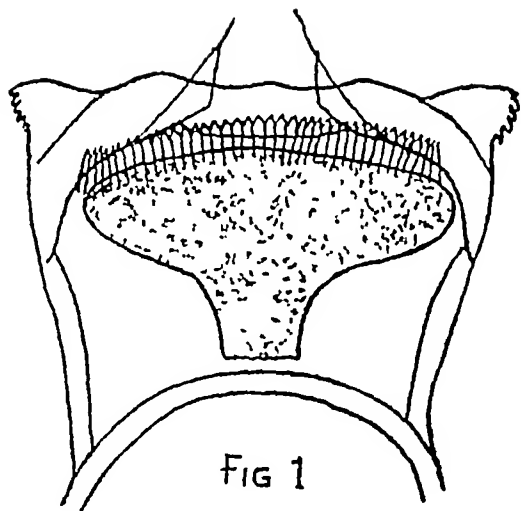


FIG 1

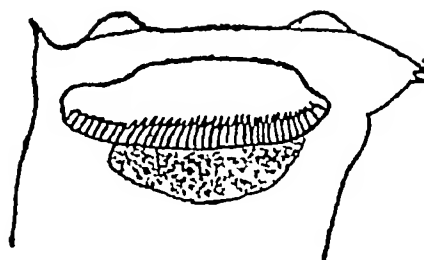


FIG.2

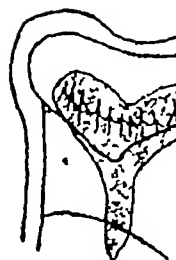


FIG 3

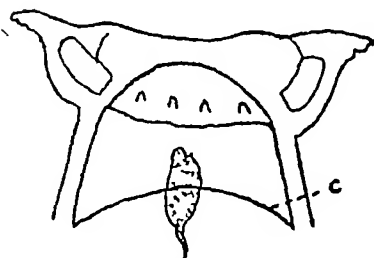
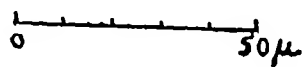


FIG. 4

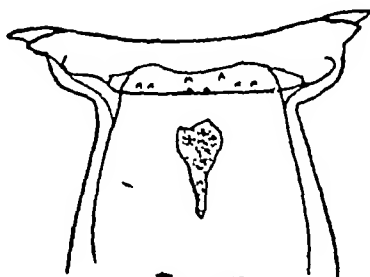


FIG. 5

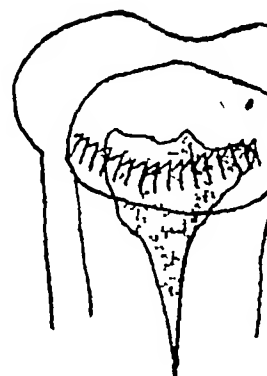


FIG. 6

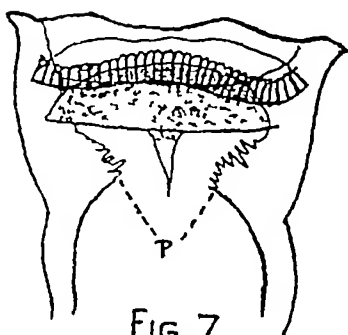


FIG. 7



FIG. 8

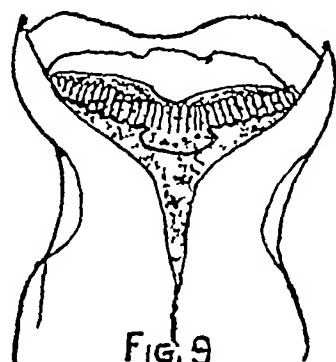


FIG. 9

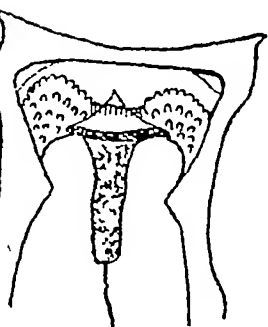


FIG. 10

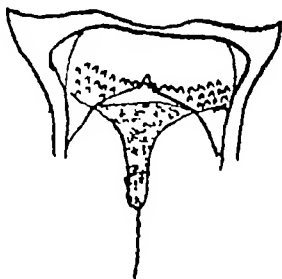


FIG. 11

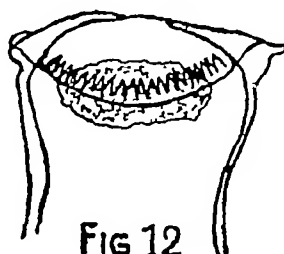


FIG 12

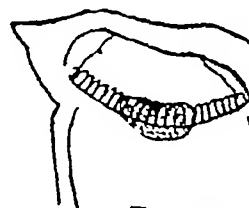


FIG 13



3 If this character is taken in conjunction with the morphology of the spermathecae (Sinton, 1927*b*), some much needed help is given in the identification of the females of the Indian *Phlebotomus*

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- |                                |  |
|--------------------------------|--|
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| SINTON, J A (1927 <i>b</i> )   | <i>Ind Jour Med Res</i> , XV, 1, pp 21—40    |

# NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*

## Part XXI.

### *PHLEBOTOMUS CHRISTOPHERSI* n sp

BY

MAJOR J A SINTON, I M S

[Received for publication, February 7, 1927]

WHILE collecting 'sandflies' at Lahore, Punjab, India, during the summer and autumn of 1923, it was noticed that a number of specimens, which superficially appeared to belong to the *minutus* group of the 'recumbent-haired' division of the Indian *Phlebotomus*, had a few erect hairs on the dorsum of the abdomen. It was thought, at first, that these hairs had been displaced at the time of capture or during subsequent handling. The abdominal hairs, however, were more profuse than those usually seen amongst the members of the *minutus* group and the palpal formula was different, although the male hypopygium was of the type seen in that group. A subsequent careful examination of these specimens showed that the spermathecae belonged to the 'crenulated' type seen in the 'erect-haired' group (Sinton, 1927a), and that the morphology of the posterior portion of the buccal cavity was quite distinct from that seen in the *minutus* group (Sinton, 1927b). It was apparent, therefore, that this was a hitherto undescribed species of Indian *Phlebotomus* for which the name *P christophersi* was proposed (Sinton, 1927a).

#### *PHLEBOTOMUS CHRISTOPHERSI* n sp (♀)

##### *General Description of Fresh Specimens*

**Naked-eye appearance** It is a medium-sized species with comparatively long legs. The general colour is greyish brown. The very dark eyes are in marked contrast to the lighter sides of the thorax.

**Microscopical appearance** No scales are present on the pleuræ. The colour varies from dark to light greyish brown. The integument of the abdomen and the dorsum of the thorax is brownish grey, while the sides of the thorax are

lighter The wings are covered with brownish grey hairs, which, in some specimens, have a distinct bluish effulgence The wing membrane has a bluish iridescence The tips of the halteres are very dark The abdominal hairs are greyish or yellowish brown and are very profuse, more so than usually seen in this position in the *minutus* group These hairs on the dorsum of the abdomen are recumbent except for small tufts of erect hairs at the posterior margins of the segments These tufts are not nearly so marked as those seen in *P papatasu*, *P major*, *P argentipes* or *P seigenti*, in all of which species the majority of the dorsal hairs are erect or semi-erect The tufts resemble those seen in *P similimus* var *hospitu* The ventral hairs are even more profuse and are not so recumbent as the majority of those seen on the dorsum The genitalia are more yellowish than the abdomen proper The antennæ are dark grey as are also the palps, except for the last segment of the latter which is a silvery white colour The legs are dark grey with silvery reflections

*Description of Stained and Mounted Specimens*

Table A gives the measurements of seven specimens of this species

The buccal cavity (Plate IX, fig 11) has four or five widely-separated and well-developed teeth The pigmented area is very small and rather carrot-shaped (Plate IX, fig 11, P) (Sinton, 1927b)

TABLE A

*Phlebotomus christophersi* n sp (♀)

Measurements in millimetres of the different parts of the body

Specimens number		1	2	3	4*	5	6	7
BODY	Olypeus and head	0 414	0 400	0 371	0 385	0 400	0 386	0 400
	Thorax	0 685	0 643	0 585	0 657	0 628	0 671	0 557
	Abdomen proper	1 285	1 643	1 500	1 428	1 428	1 571	1 357
	Sup clasper, seg 1	0 143	0 143	0 143	0 171	0 143	0 171	0 143
	TOTAL LENGTH	2 5	2 83	2 6	2 64	2 6	2 8	2 45
LABIUM		0 243	0 257	0 235	0 257	0 271	0 285	0 247
ANTENNA	Segment III	0 143	0 143	0 114	0 128	0 135	0 143	0 135
	Segment IV	0 078	0 085	0 071	0 071	0 071	0 078	0 071
	Segment V	0 078	0 085	0 071	0 071	0 071	0 078	0 071
	Segment VI	0 078	0 085	0 071	0 071	0 071	0 078	0 071
	Segments XII—XVI	0 271	0 285	0 257	0 257	0 257	0 285	0 261
	TOTAL LENGTH	1 140	1 200	1 030	1 100	1 085	1 185	1 100

\* Type specimen

TABLE A—*contd*

Specimens number		1	2	3	4 *	5	6	7
PALP	Segment 1	0 030	0 036	0 033	0 033	0 036	0 036	0 036
	Segment 2	0 090	0 090	0 093	0 099	0 090	0 093	0 087
	Segment 3	0 180	0 168	0 156	0 171	0 180	0 180	0 180
	Segment 4	0 114	0 111	0 105	0 111	0 123	0 126	0 111
	Segment 5	0 255	0 270	0 240	0 329	0 240	0 300	0 255
	TOTAL LENGTH	0 678	0 675	0 627	0 738	0 669	0 744	0 669
WING	Length	1 730	1 714	1 570	1 840	1 657	1 714	1 571
	Breadth	0 430	0 414	0 370	0 400	0 371	0 428	0 357
	$\alpha$	0 200	0 200	0 157	0 214	0 185	0 185	0 185
	$\beta$	0 300	0 300	0 307	0 285	0 328	0 343	0 285
	$\gamma$	0 328	0 314	0 285	0 285	0 271	0 271	0 271
	$\delta$	0 085	0 071	0 071	0 085	0 057	0 057	0 071
	$\epsilon$	0 357	0 357	0 314	0 357	0 328	0 357	0 357
	$\theta$	0 800	0 785	0 743	0 785	0 814	0 814	0 771
HIND LEG	Femur	0 757	0 757	0 700	0 743	0 743	0 771	0 714
	Tibia	1 043	1 000	0 900	0 957	0 985	1 028	0 943
	Tarsus, seg 1	0 528	0 530	0 485	0 514	0 493	0 543	0 500
	Tarsus, segs 2—5	0 628	0 628	0 600	0 614	0 600	0 657	0 571
	TOTAL LENGTH †	3 00	2 9	2 7	2 8	2 8	3 0	2 7

\* Type specimen

† Not including coxa and trochanter

The *body* averages about 2.6 mm in length when extended. It is almost as long as the hind leg and is about 1.6 times the length of the wing.

The *antennae* (Plate IX, figs 4 and 5). The IIIrd antennal segment is equal to about half the combined lengths of segments XII to XVI, but is slightly shorter than the lengths of segments IV to V. The distal end of the Vth segment reaches the distal end of the proboscis. The total length of the antenna is 4.0 to 4.2 times the length of segments XII to XVI combined, 8 to 9 times that of segment III, about 0.4 times that of the hind leg and 0.65 to 0.70 that of the wing. The geniculate spines are stout and well-developed (Plate IX, figs 4 and 5). Those on segments XIV and XV reach to the bases of the succeeding segments (Plate IX, fig 5). The antennal formula is 2 over III to XV.

The *palps* (Plate IX, fig 3). The palpal formula is 1, 2, 4, 3, 5, the relative lengths of the segments averaging 3.1, 8.0, 15.3, 10, 23.3. This is different from

*P. minutus*, which has a similar formula, but in which the IVth segment nearly equals the third. The IIIrd segment is markedly incrassate in its proximal third, and the incrassation carries a very large number, about 50, of the 'curious modified spines' described by Newstead (Plate IX, fig 3, N). The ratio palp over labium is 2.6 to 2.8.

The wings (Plate IX, fig 1). The ratio length over breadth is 4.0 to 4.4. The length is about 0.58 times that of the hind leg. The ratio  $\alpha$  over  $\beta$  averages about 0.62 (0.51 to 0.75),  $\beta$  is approximately equal to  $\gamma$ ,  $\alpha$  over  $\gamma$  averages about 0.64 (0.55 to 0.75),  $\delta$  over  $\alpha$  averages about 0.37 (0.31 to 0.45),  $\alpha$  over  $\epsilon$  averages 0.55 (0.55 to 0.60),  $\theta$  over  $\epsilon$  2.28 (2.16 to 2.50),  $\alpha + \beta$  over  $\theta$  about 0.64 and over wing length about 0.47 (0.46 to 0.49).

The hind leg is about 2.7 to 3.0 mm in length. The relative lengths of the different segments of the leg averaged 14.5, 19, 10, 12.

The genitalia. The spermathecae (Plate IX, fig 9) are sausage-shaped and have 8 or 9 crenulations. There are four spines on the post-genital ridge. These structures and the furca have already been described and figured in another paper of this series (Sinton, 1927a).

#### *Diagnostic Features of the Female*

The erect hairs on the dorsum of the abdomen and the crenulated spermathecae at once differentiate this species from all the members of the 'recumbent-haired' group. The palpal formula 1, 2, 4, 3, 5 is also different from any of the members of this group recorded from India except *P. minutus* type. As pointed out above, the fact that the IIIrd segment of the palp is  $1\frac{1}{2}$  times as long as the IVth in *P. christophersi* distinguishes this species from *P. minutus* type in which these segments are nearly equal in length.

Amongst the 'erect-haired' group the palpal formula in *P. papatasi* is 1, 4, 2, 3, 5, in *P. major* 1, 4 (2, 3), 5, in *P. argentipes* 1, 4, 2, 3, 5, in *P. sergenti* 1 (4, 2), 3, 5 and in *P. simillimus* var *hospiti* 1, 2, 3, 4, 5, while in *P. christophersi* it is 1, 2, 4, 3, 5.

The morphology of the spermathecae is distinctly different from that found in any of the other Indian members of this group except *P. simillimus* var *hospiti* (vide Sinton, 1927a), while the shape of the posterior portion of the buccal cavity seems to be characteristic of this species (Sinton, 1927b).

#### PHLEBOTOMUS CHRISTOPHERSI n sp. (♂)

##### *General Description of Fresh Specimens*

Naked-eye appearance. The insect resembles a large male of the *minutus* group. The colour is dark grey.

Microscopical appearance. The appearance is more or less the same as that described in the female, except that the number of erect hairs on the dorsum of the abdomen is much fewer, indeed these may be so scanty that they are missed frequently in the examination of fresh specimens. The very profuse nature of the recumbent hairs on the abdomen enables one to differentiate roughly the males of this species from those of *P. minutus* and its varieties in which these hairs are scanty.

## Description of Stained and Mounted Specimens

Table B gives the measurements of four specimens of this insect

TABLE B.

*Phlebotomus christophersi* n sp (♂)

Measurements in millimetres of the different parts of the body

Specimens number		1 *	2	3	4	5
BODY	Clypeus and head	0 371	0 385	0 371	0 428	0 428
	Thorax	0 600	0 540	0 557	0 630	0 643
	Abdomen proper	1 500	1 328	1 257	1 314	1 300
	Sup clasper, seg 1	0 240	0 234	0 235	0 240	0 255
	TOTAL LENGTH	2 7	2 5	2 4	2 6	2 6
LABIUM		0 214	0 214	0 214	0 230	0 230
ANTENNA	Segment III	0 150	0 140	0 157	0 154	
	Segment IV	0 080	0 079	0 085	0 085	
	Segment V	0 080	0 079	0 085	0 085	
	Segment VI	0 080	0 079	0 085	0 085	
	Segments XII—XVI	0 271	0 257	0 285	2 080	
	TOTAL LENGTH	1 143	1 100	1 214	1 190	
PALP	Segment 1	0 030	0 033	0 033	0 030	0 030
	Segment 2	0 084	0 081	0 084	0 093	0 090
	Segment 3	0 156	0 144	0 156	0 156	0 153
	Segment 4	0 114	0 105	0 111	0 123	0 120
	Segment 5	0 270	0 234	0 255	0 300	0 270
	TOTAL LENGTH	0 654	0 597	0 634	0 702	0 663
WING	Length	1 500	1 500	1 630	1 570	1 570
	Breadth	0 357	0 357	0 370	0 370	0 370
	α	0 143	0 157	0 143	0 057	
	β	0 271	0 243	0 285	0 364	..
	γ	0 314	0 300	0 328	0 364	.
	δ	—0 014	0 028	0 000	—0 114	.
	ε	0 271	0 285	0 300	0 185	
	θ	0 657	0 643	0 714	0 685	

\* Type specimen

TABLE B—*contd*

Specimens number		1 *	2	3	4	5
Hind Leg	Femur	0 085	0 028	0 700	0 700	
	Tibia	0 014	0 814	0 900	0 928	
	Tarsus, seg 1	0 485	0 444	0 500	0 500	
	Tarsus, segs 2—5	0 614	0 543	0 630	0 614	
	TOTAL LENGTH †	2 7	2 4	2 6	2 74	
Sup. clasper	seg 1	0 240	0 234	0 234	0 240	0 255
	seg 2	0 114	0 108	0 114	0 114	0 120
Intermediate appendage		0 204	0 189	0 165	0 210	0 219
Intromittent organ		0 057		0 055	0 057	0 055
Inferior clasper		0 234	0 210	0 225	0 240	0 255
Sub genital lamellæ		0 204	0 195	0 165	0 204	0 225

\* Type specimen

† Not including coxa and trochanter

The *buccal cavity* (Plate IX, fig 10) has two or three large teeth and a number of much smaller ones. The pigmented area is carrot-shaped as in the female (Sinton, 1927b).

The *body* averages about 2.5 mm in length when extended. It is not quite as long as the hind leg and is about 1.6 times the wing length. The scars of a very few erect hairs may be present on the 2nd abdominal segment but not on the succeeding ones.

The *antennæ* (Plate IX, figs 6 and 7). The length of the IIIrd segment is slightly greater than half the combined lengths of segments XII to XVI, but is shorter than the lengths of segments IV and V combined. The total length of the antenna is about 4.25 times the lengths of segments XII to XVI combined, about 7.7 times that of segment III, about 0.43 times that of the hind leg and 0.75 that of the wing length. The geniculate spines are well-developed (Plate IX, figs 6 and 7). The spines on segments XIV and XV reach to the bases of the succeeding segments. The antennal formula is 1 over III to XV.

The *palps* (Plate IX, fig 8). The palpal formula is 1, 2, 4, 3, 5, the relative lengths of the segments averaging 2.7, 7.5, 13.5, 10, 23.5. The IIIrd segment has not the very marked incrustation seen in the female and the 'curious modified spines' are only about 8 to 10 in number. The ratio palp over labium is 2.8 to 3.0.

The *wings* (Plate IX, fig 2). The ratio length over breadth is about 4.25. The length is about 0.58 times that of the hind leg. The venation differs from that of the female wing in that  $\delta$  is small or even negative as in (Plate IX,

fig 2), which is rather like the venation of *P. signatipennis* in this particular\*. The ratio  $\delta$  over  $\alpha$  varied from  $-2.0$  to  $+0.18$ . This smallness of  $\delta$  is in some cases accompanied by a comparatively short  $\alpha$ . The ratio  $\alpha$  over  $\beta$  averaged  $0.45$  ( $0.15-0.64$ ),  $\beta$  over  $\gamma$  averaged  $0.89$  ( $0.8-1.0$ ),  $\alpha$  over  $\gamma$  averaged  $0.39$  ( $0.15-0.52$ ),  $\alpha$  over  $\epsilon$   $0.46$  ( $0.31-0.55$ ),  $\theta$  over  $\epsilon$   $2.7$  ( $2.25-3.7$ ),  $\alpha+\beta$  over  $\theta$   $0.61$  ( $0.60-0.63$ ) and  $\theta$  over wing length  $0.43$ .

The hind leg is about  $2.4$  to  $2.7$  mm in length. The relative lengths of the segments averaged  $14, 18.4, 10, 12.4$ .

The genitalia are of the *minutus* type (Plate IX, fig 12). The relative lengths of the 1st and 2nd segments of the superior clasper, the intermediate appendage, the inferior clasper and the sub-genital lamellæ are  $22.2, 10, 18.3, 21.6, 18.3$ , respectively. From these figures it is seen that the first segment of the superior clasper and the inferior clasper are almost equal in length and that the lengths of the intermediate appendage and the sub-genital lamellæ are equal. The spines on the distal segment of the superior clasper are about  $90 \mu$  in length, while the small non-deciduous hair is about half this length. There is a very distinct narrow fissure in the intermediate appendage commencing on its ventral margin at about one-third of the length of the organ from its apex (Plate IX, fig 12, C). The genital filaments are protruded for about  $60$  to  $70 \mu$ . The ends of the filaments are curved upwards and outwards, but not so markedly as in the case of *P. squamipleuris* nor have they the bulbous ends seen in that species.

#### Diagnostic Features of the Male

The scantiness of the erect hairs on the dorsum of the abdomen in this species make this feature of little diagnostic value. None of the other Indian species in the 'erect-haired' group, except *P. sergenti*, *P. squamipleuris* and *P. similis* var. *hospiti*, have four spines on the distal segment of the superior clasper. The distribution of these spines in *P. sergenti* is markedly different from that seen in *P. christophersi* (cf Sinton, 1924, Plate XLV, fig 6). The genitalia of the other two species mentioned closely resemble that seen in *P. christophersi* but neither of these species have a palpal formula of  $1, 2, 4, 3, 5$ . In the case of *P. squamipleuris* the peculiar morphology of the genital filaments and the absence of geniculate spines on the IVth antennal segment at once differentiate this species. In the case of *P. similis* var. *hospiti* the wing is much broader, the ratio length over breadth being about  $3.36$ , while in *P. christophersi* the wing is relatively much narrower, the ratio being about  $4.25$ . The morphology of the buccal cavity is also characteristic.

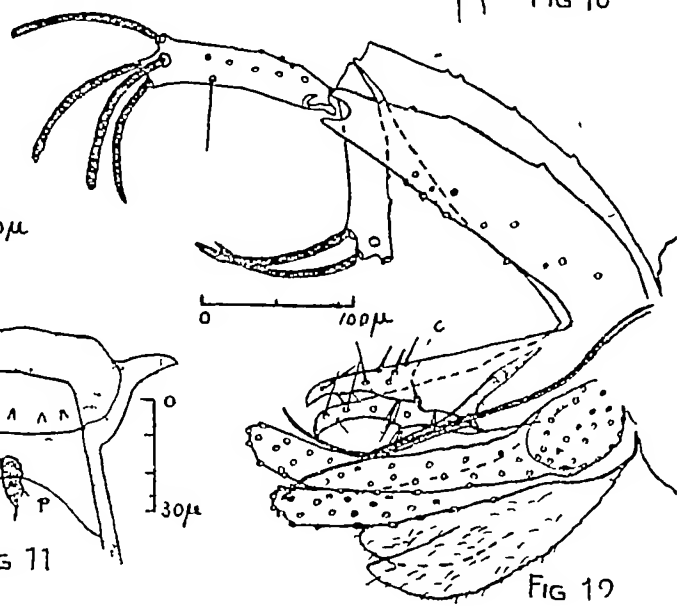
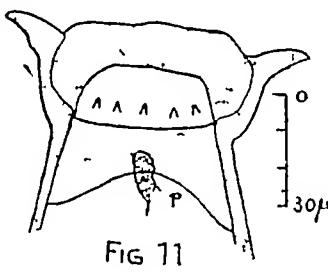
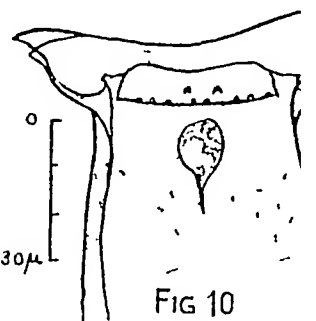
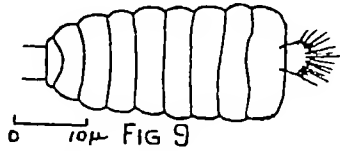
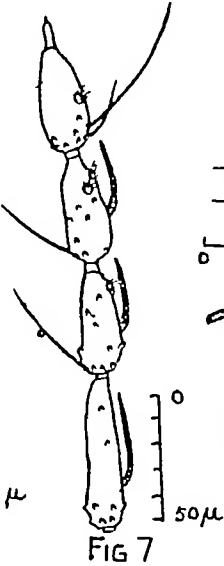
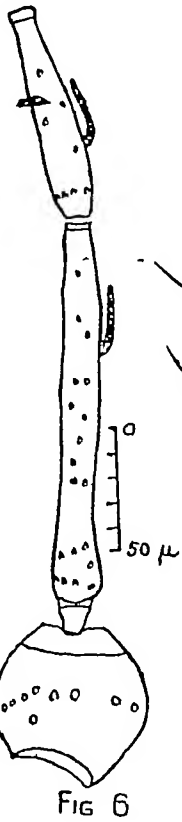
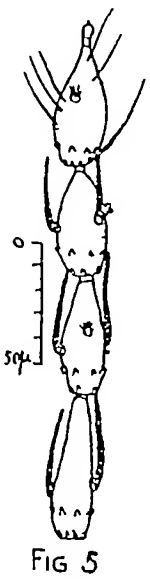
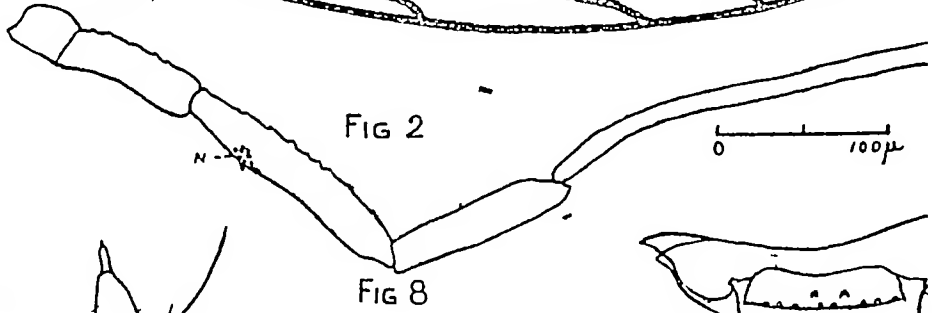
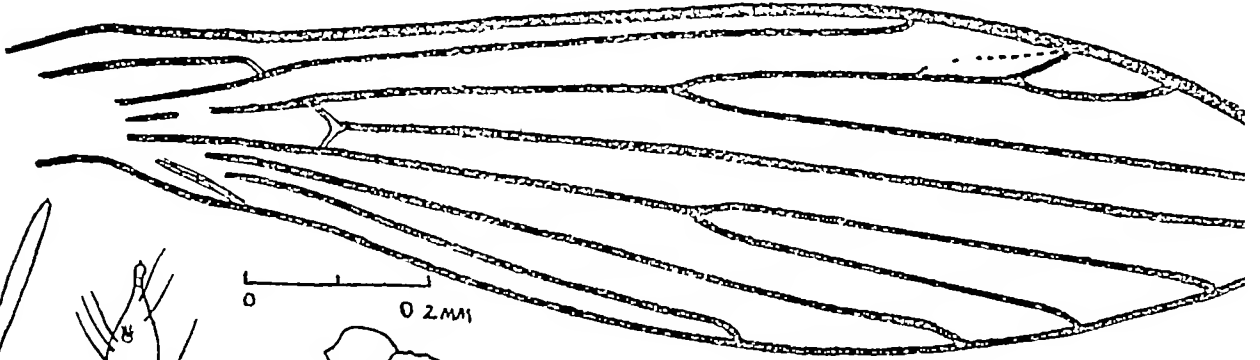
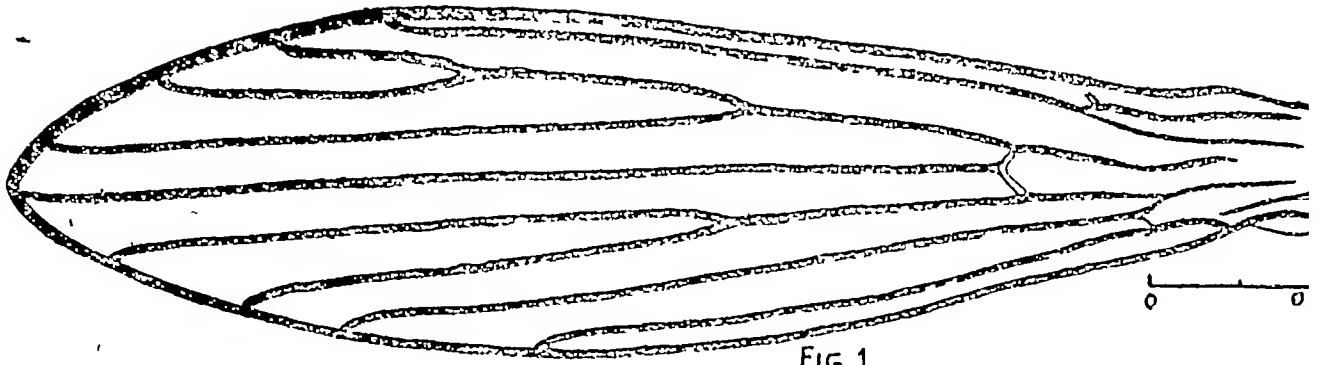
In the 'recumbent-haired' group the hypopygium of *P. minutus* and its varieties closely resembles that of *P. christophersi*, but in none of this group except in *P. minutus* type has a palpal formula of  $1, 2, 4, 3, 5$  been described. In *P. minutus* type the IIIrd palpal segment is almost equal to the IVth, which is not the case in *P. christophersi*. The very hairy abdomen in the latter species also helps to differentiate it from the former.

\*The dotted line in Plate IX fig 2, shows the more common position of the upper branch of the 2nd fork of the second vein of the wing.



DESCRIPTION OF PLATE IX  
*Phlebotomus christophersi* n sp

- Fig 1 Wing (♀)  
„ 2 Wing (♂)  
„ 3 Palp (♀) N are the 'curious modified spines'  
„ 4 Segments II, III and IV of the antenna (♀)  
„ 5 Segments XIII to XVI of the antenna (♀).  
„ 6 Segments II, III and IV of the antenna (♂)  
„ 7 Segments XIII to XVI of the antenna (♂)  
„ 8 The palp (♂)  
„ 9 The spermatheca (♀)  
„ 10 Posterior portion of the buccal cavity (♂)  
„ 11 Posterior portion of the buccal cavity, showing the teeth and the pigmented area (P) (♀)  
„ 12 The male hypopygium C is the fissure in the intermediate appendage



## DISTRIBUTION

This species formed about 3 per cent of the 'sandfly' catch made at the Central Jail, Lahore, Punjab, during August and the early half of September 1923. One female specimen was captured in Lahore Cantonment during May 1921, and two specimens in a railway carriage at Kalka Railway Station, Punjab, in June 1923.

## CONCLUSION

The species here described differs, more especially in the female, from any of the species of *Phlebotomus* hitherto described and the name *P. christophersi* is proposed for it.

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- |                       |   |
|-----------------------|---|
| SINTON, J. A. (1924)  | <i>Ind Jour Med Res</i> , XI, 3, pp 807—815 |
| SINTON, J. A. (1927a) | <i>Ind Jour Med Res</i> , XV, 1, pp 35—40   |
| SINTON, J. A. (1927b) | <i>Ind Jour Med Res</i> , XV, 1, pp 29—40   |

# AN EXPERIMENTAL INVESTIGATION INTO THE ACTION OF ORGANIC COMPOUNDS OF ANTIMONY

BY

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IN a preliminary note regarding the pharmacological action of the organic-antimony derivatives (aromatic), I pointed out that a number of these new derivatives had been introduced for the treatment of tropical diseases and were being largely used in India. The starting point of all these compounds was sodium-para-amino-phenyl-stibinate (or sodium p-stibanilate), which is the antimony analogue of atoxyl or sodium p-arsanilate from which all the organic arsenicals were originated. This compound was given the name of 'Stibamin' by Brahmachari from the analogy to the corresponding compound of arsenic called 'Arsamin' (Atoxyl). Stibamin, however, is not a very stable compound and its therapeutic applicability for that reason is limited. To get a more stable derivative an acetyl group was introduced into it and sodium acetyl-p-amino-phenyl-stibinate known as *stibacetin* was prepared. This was the first aromatic compound of antimony to be used in the treatment of leishmaniasis, and was introduced under the trade name of 'Stibenyl'. This compound, although it gave good results in Europe was found uniformly to be of no value in the treatment of leishmaniasis in this country. Brahmachari prepared a carbamide derivative of p-amino-phenyl-stibinic acid and the compound which is now known as *urea-stibamine* came into existence. The combination of urea with p-amino-phenyl-stibinic acid renders the resultant compound not only more stable but also more soluble and more readily diffusible into the tissues. This compound has given excellent results in the treatment of kala-azar, it is also sold under the trade name of 'Stiburea' by the Union Drug Company of Calcutta. Von Heyden a few years ago prepared sodium meta-chloro-para-acetyl-amino-phenyl stibinate (*von Heyden* '471') which was tried by Napier in the treatment of kala-azar. This compound has proved very effective and has been put on the market under the trade name 'Stibosan'. Other

therapeutically active compounds which have been introduced are '*Neostam*' or *stibaminic glucoside* (Burroughs Wellcome), '*amino-stiburea*' (p-amino-phenylstibinic acid urea glucose), and *von Heyden* '693' (amine salt of p-amino-phenylstibinic acid) The last named compound has been found by Napier to be the most effective and the least toxic of all the organic compounds used in the treatment of kala-azar

Although most of these compounds have been largely used intravenously in the treatment of leishmaniasis no attempt has been made to work out their pharmacological action and explain some of the acute symptoms resembling 'nitritoid crises,' which are sometimes produced after their intravenous use Further, the mode of action of these compounds is not known In the following experiments an attempt has been made to investigate these points

In all our experiments we used healthy cats weighing about 2 to 2½ kilos in weight Urethane intramuscularly was used as an anæsthetic, a small quantity of ether being given whenever necessary The blood pressure was recorded by a cannula in the carotid artery, the respirations were recorded by means of a tambour connected with the side tube of the tracheal cannula The pressure in the pulmonary artery and the venous pressure in the inferior vena cava were recorded by a modification of the delicate method described by Jackson and Rapp and the volume of different organs by means of oncometers The drugs were injected into the femoral vein in doses ranging from 10 mg to 100 mg in 4 per cent solutions, but 2 per cent solutions were used in the case of antimony tartrates Doses below 50 mg gave slight reactions, doses of 50 mg or more gave marked reactions in an animal of average size, while with 100 mg the effects were very marked amounting to toxic effect

### ACTION ON CIRCULATION

Graph I, fig (a) shows the effect of intravenous injections of urea-stibamine It will be seen that 25 mg in a cat of average size produce little or no effect on the blood pressure or respiration A 100 mg dose (in 4 per cent solution) in the same animal is followed by a very large and persistent fall of blood pressure specially if the injection is given rapidly The marked fall in the systemic blood pressure is a feature of all organic derivatives of antimony, with antimony tartrates the fall is not nearly as marked even when very large doses are given The fall of the systemic pressure is probably partly due to the lessened output of the left ventricle and partly to the dilatation of the vessels of some parts of the splanchnic area Jackson and Smith have suggested that the initial fall in systemic blood pressure is due, in the case of salvarsan, to the alkalinity of the solution which irritates the endocardium resulting in temporary weakness and dilatation of the heart The hydrogen-ion-concentration of antimony compounds ranges between pH 4 and 7 and it may be argued that the acidity of the solutions is responsible for the large initial fall shown in the accompanying Graph [figs (a) and (b)] If this were so, the fall would be more marked with solutions of antimony tartrates which are much more acid than those of the other organic compounds, but this is not the case The blood pressure gradually recovers and regains its normal level



1



Vaguet



1

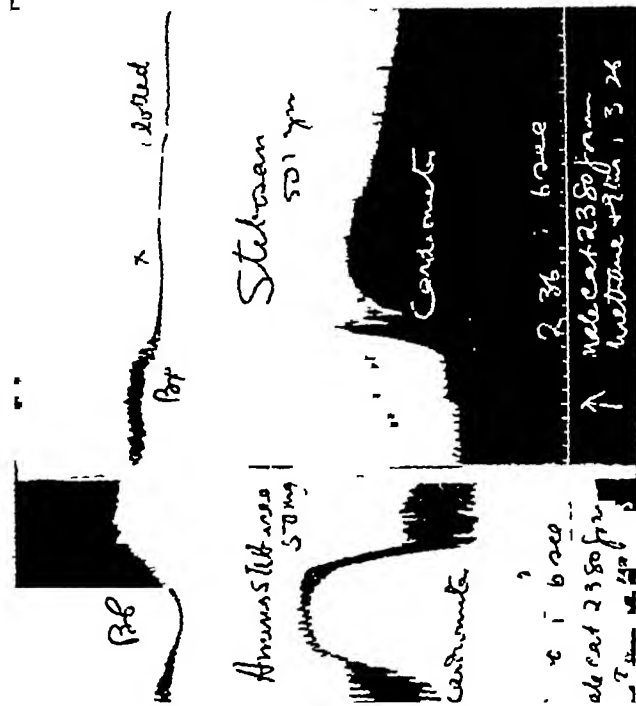
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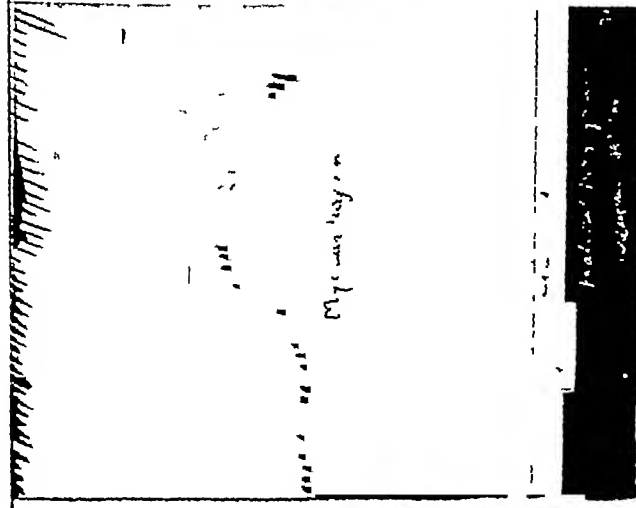
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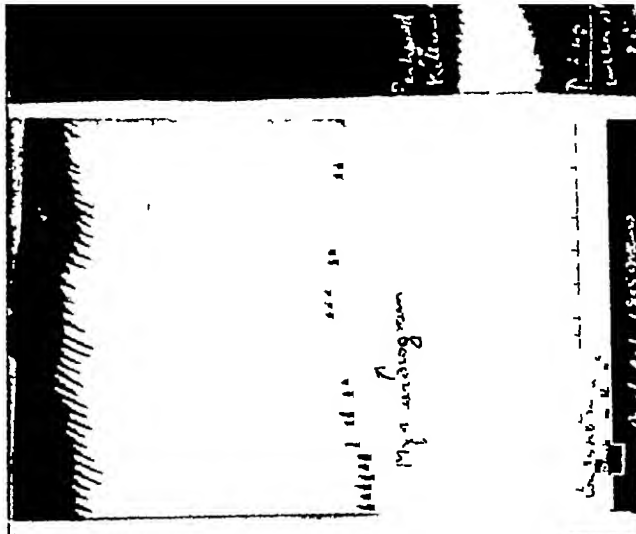
GRAPH II.



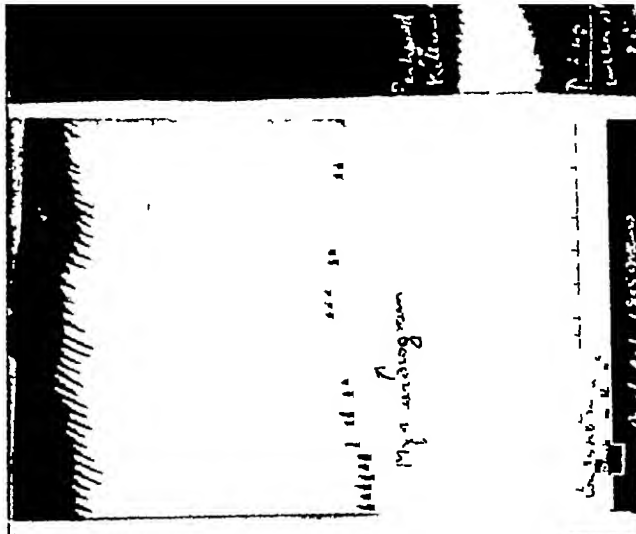
(a)



(b)



(c)



(d)

in quarter of an hour or more Fig (b) shows the effect of 100 mg in another animal, the stoppage of respiration has lasted for even a longer time Fig (c) shows the effect of 25 mg of urea-stibamine in an animal in which the vagi have been cut It will be seen that here the fall of pressure is as marked as with 100 mg and the respirations show no preliminary stopping but a well marked stimulation Constriction of the bronchi is also noticed

Graph II, figs (a) and (b) show the effect of 50 mg of '*amino-stiburea*' and '*stibosan*' on the heart volume as recorded by a cardiometer It will be observed from the rise in the cardiometer tracing that the heart is considerably dilated, the movements have become very weak and the amplitude is markedly decreased This dilatation is very persistent and is due to the great rise in the pulmonary pressure which causes the right ventricle to dilate The depressing effects on the heart last for a considerable time [fig (b)] but eventually the organ may regain its normal force and rhythm Figs (c) and (d) show the record of the contraction of ventricles as taken by a Cushney's myocardiograph Sodium antimony tartrate and urea-stibamine both produced a well marked and persistent depression of this organ, the force of contraction and the amplitude of the movements both being decreased The dilatation of the heart is not seen in this tracing but the weakening effect on the heart is visible and this corresponds to the fall in the systemic pressure Fig (e) shows the effect of perfusion of an isolated heart with urea-stibamine and the well marked depression which is produced The heart recovers after a time but not entirely when the perfusion is stopped

*Pulmonary and venous pressure*—Graph III shows the effect of large doses of the antimony compounds on the systemic and pulmonary blood pressures It will be noted that while the blood pressure in the carotid falls, the pulmonary pressure shows a considerable and persistent rise Fig (a) shows that in the case of sodium antimony tartrate the pulmonary pressure does not rise synchronously with the fall of the carotid pressure The rise in the pulmonary pressure comes on some time later, and persists till after the carotid pressure has regained its normal level Even small doses of antimony tartrates, such as 20 to 25 mg produce an appreciable rise in the pulmonary pressure Figs (b) and (c) show that the pulmonary pressure rises abruptly as soon as the carotid pressure falls, it keeps at this high level for a time and then gradually returns to normal In Fig (d) 100 mg of '*amino-stiburea*' produces only a slight rise of carotid pressure and yet there is a marked rise in the pulmonary pressure Smaller doses of organic antimony compounds have similar effects but the rise of pressure is correspondingly less It will be seen that when the pulmonary pressure rises, the excursions of the heart and respirations are no longer seen on the curve This appears to be due to the fact that when the pulmonary pressure is high, the arterioles in the lungs are put on such high tension that regular respiratory movements of the lungs are not sufficient to cause any change in the pulmonary vessels

These changes are very similar to those described by Jackson and Smith (1918) in the case of salvarsan The acidity of the solutions may, to some extent, be responsible, but there is no doubt that the action of the antimony ion is mainly responsible for the rise The pulmonary pressure in the case of antimony compounds



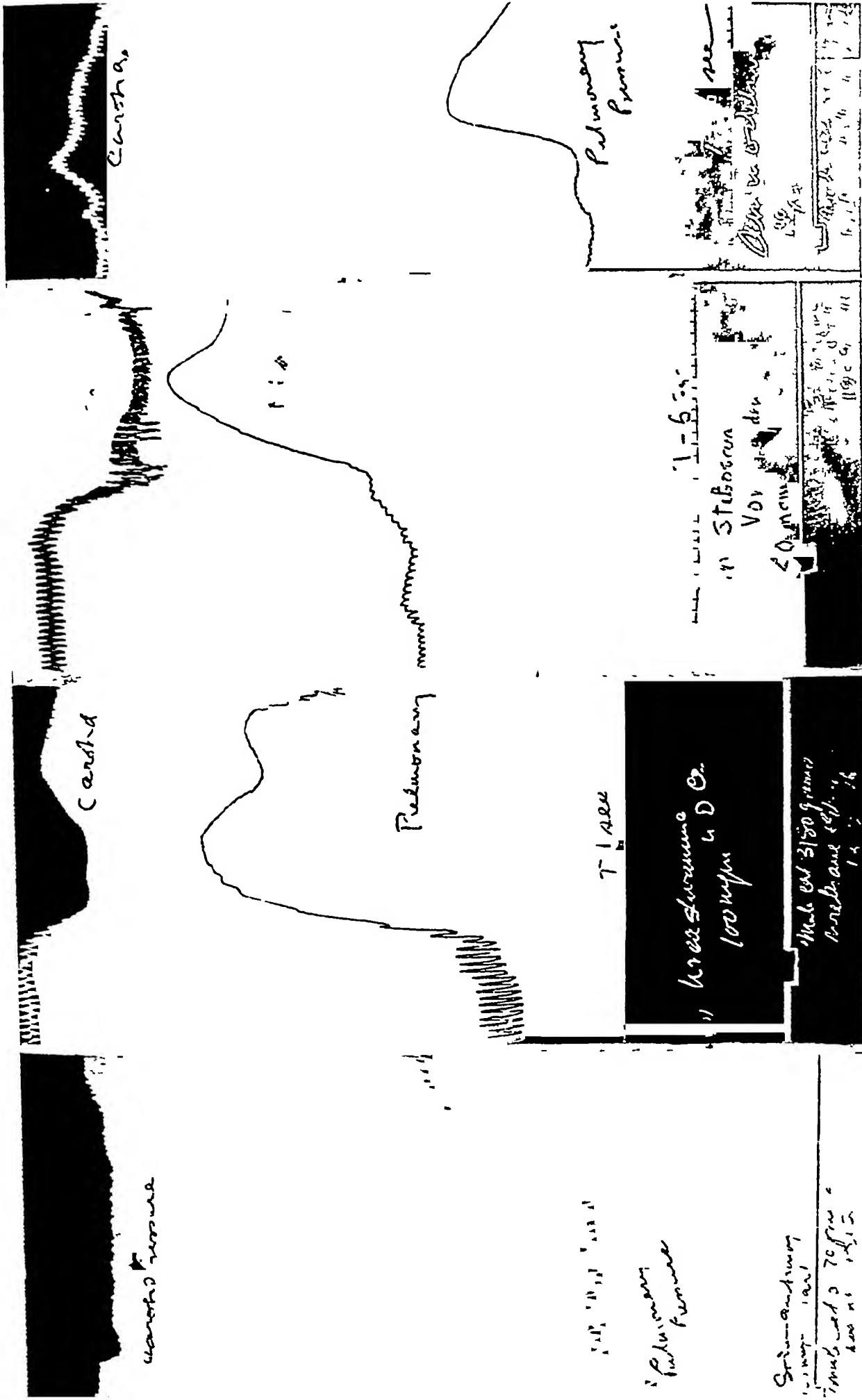
risers 100 per cent or more while at the same time a marked fall in the systemic pressure is produced. This means that while the right side of the heart is doing more than double the amount of its usual work, the left side is doing considerably less than its normal amount. The blood accumulates in the right side of the heart owing to progressively increasing obstruction in the lungs and the right ventricle dilates [Graph II, figs (a) and (b)]. The pressure in the vena cava remains unchanged as long as compensation is maintained. When the strain on the right side is too much and the dilatation is extreme, the systemic pressure falls and the venous pressure rises. We have noted in our experiments that small doses such as 20 to 25 mg of the antimony compounds caused an appreciable rise in the pressure in the inferior vena cava as recorded by a saline manometer. This, however, is soon compensated. With large doses when the changes in the heart and in the pulmonary and venous pressure are great and persistent, the heart passes into a condition of fibrillation and in some cases the animals die of delirium cordis.

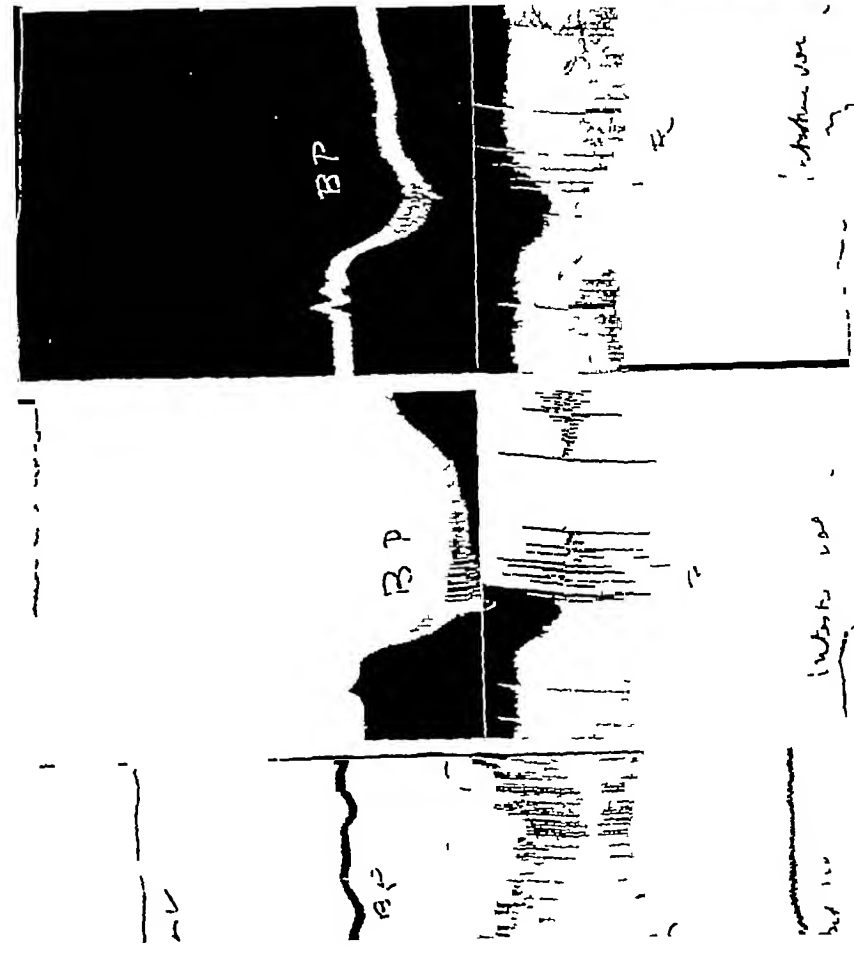
#### ACTION ON THE ABDOMINAL ORGANS

Graph IV, figs (a), (b) and (c) show the effect of 100 mg of sodium antimony tartrate, stibosan and urea-stibamine on the blood pressure, respiration and intestine and limb volumes. It will be observed that the effect of the tartrate on the blood pressure is not so marked as in the case of '*stibosan*' and '*urea-stibamine*'. The limb volume shows only a slight fall corresponding to the fall in the blood pressure. The intestinal volume shows a slight preliminary fall corresponding to the fall in the blood pressure, but this is soon followed by a slight but definite rise showing that there is dilatation of the blood vessels in this area. The peristaltic movements become increased and irregular. In figs (d) and (e), instead of the intestinal volume, the kidney volume has been recorded. There is well marked decrease in the volume of this organ and the rhythmic contractions become more apparent.

Graph V shows the effect of sodium antimony tartrate and a number of organic antimony compounds on the spleen volume. It will be noticed that in the case of all these compounds a dose of 50 mg, which produces little disturbance of blood pressure and respiration, produces a well marked increase in the volume of the spleen. This dilatation of the spleen is not so marked in the case of antimony tartrates but even with small doses of the organic compounds the effect is well marked and persistent. Graph VI, fig (a) shows the effect of repeated injections of *stibosan*, '*amino-stiburea*' and '*urea-stibamine*' on the spleen. Not only does this organ show a tremendous increase in its volume but the rhythmic movements are very markedly stimulated. Fig (b) shows the record of liver volume. It will be seen that while the blood pressure has fallen the volume of this organ has considerably increased.

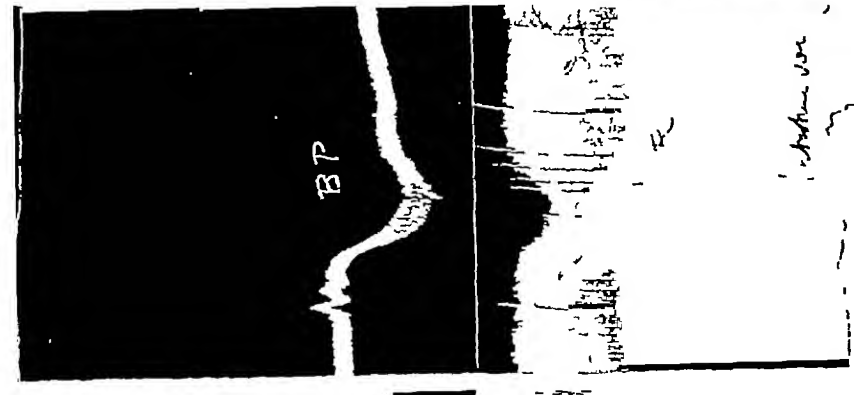
It is possible that increase in the volume of these organs may be due to a specific effect of antimony on the arterioles. It will be seen, however, that the kidney usually contracts while the spleen and the intestines dilate. The cause of these variable reactions of the internal organs is not quite clear but it is probable that both central and peripheral influences are at work in producing these reactions.





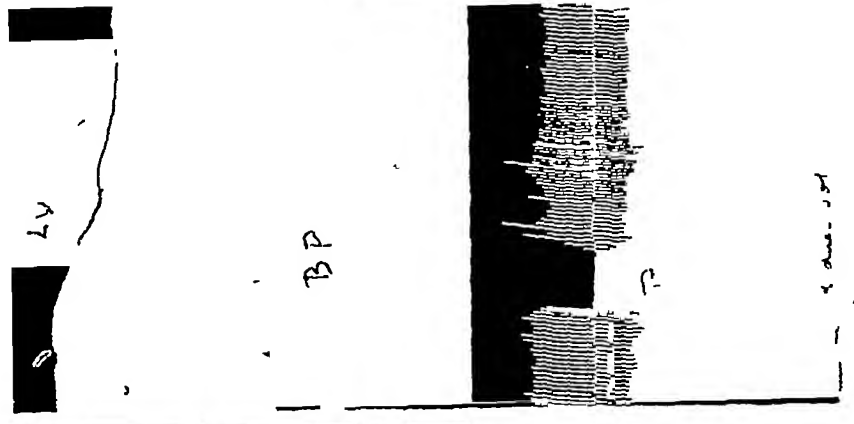
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Silberau  
Vom Wasser  
50 m



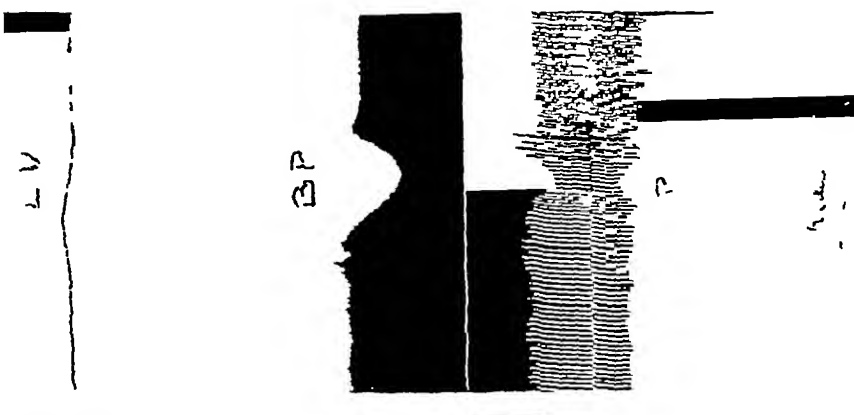
1 24 T 6500

breitste Baum  
(Praktisch alle)  
50 m



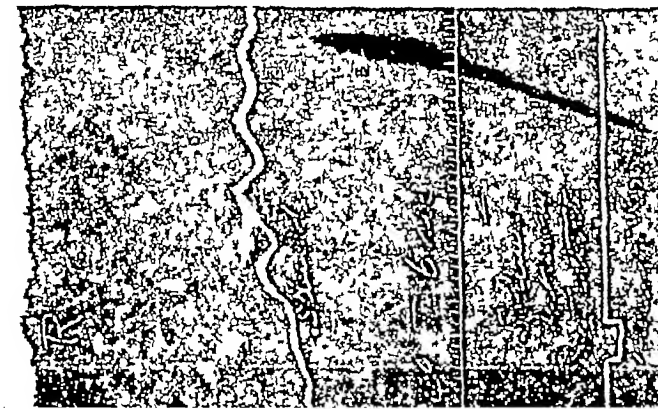
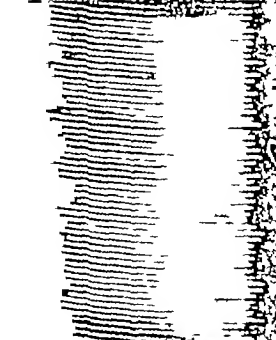
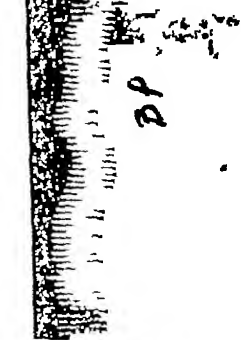
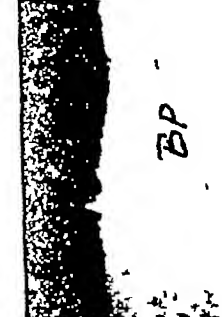
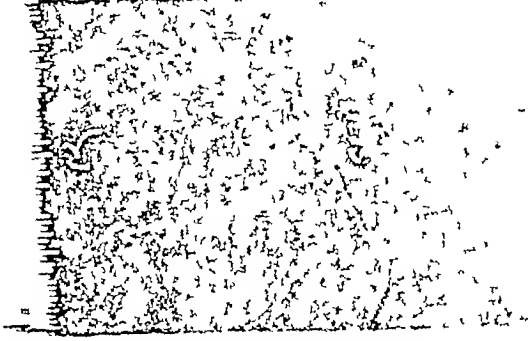
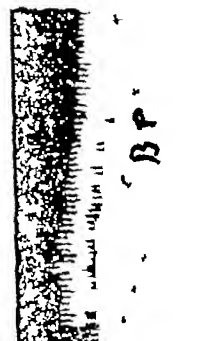
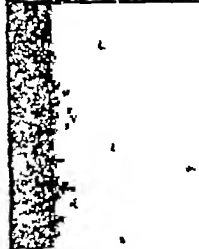
7 4 T 6500

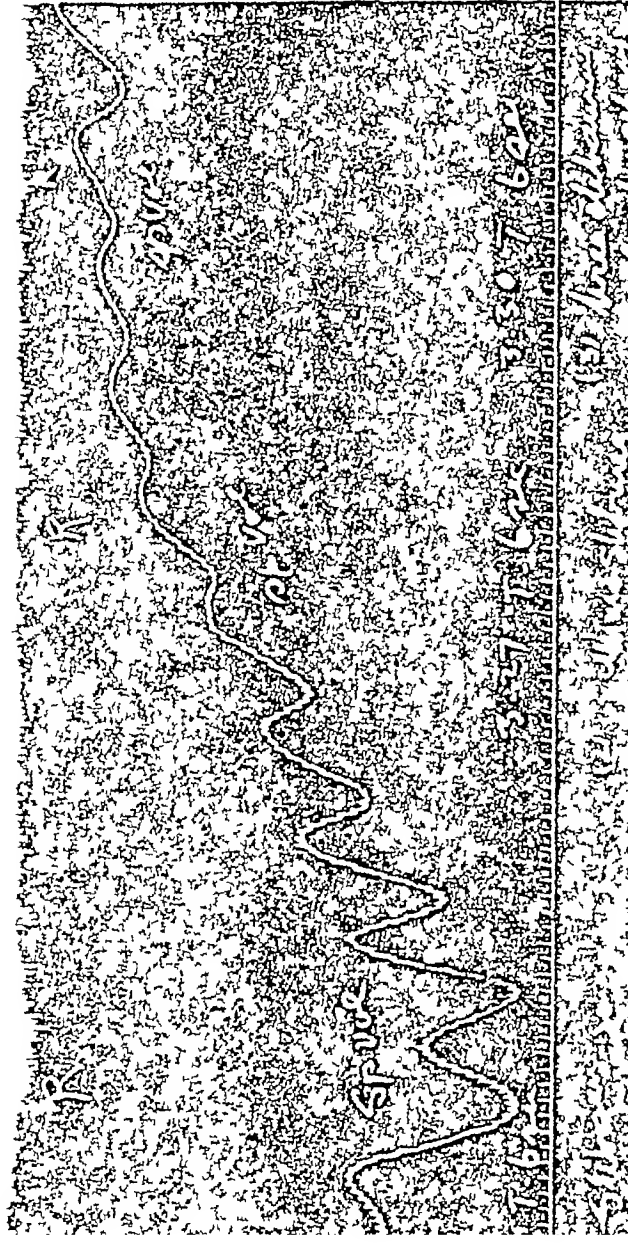
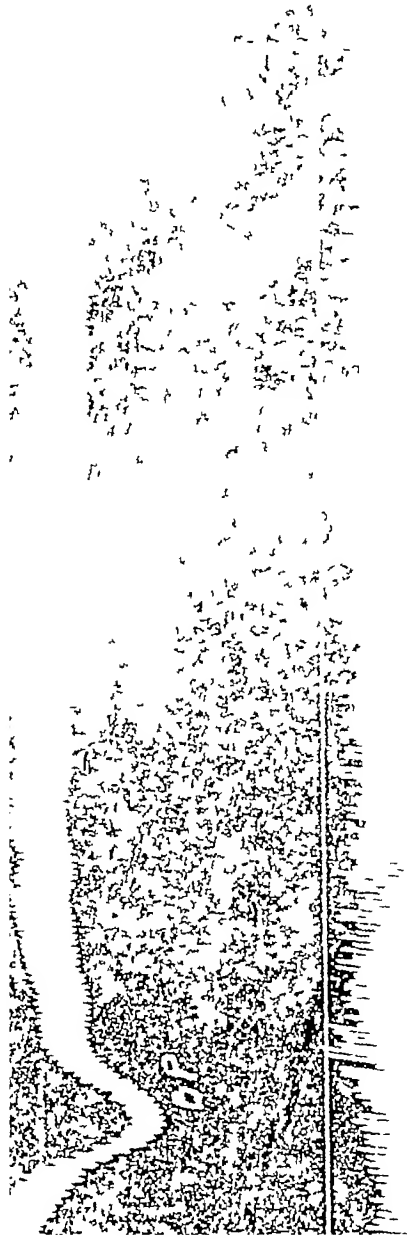
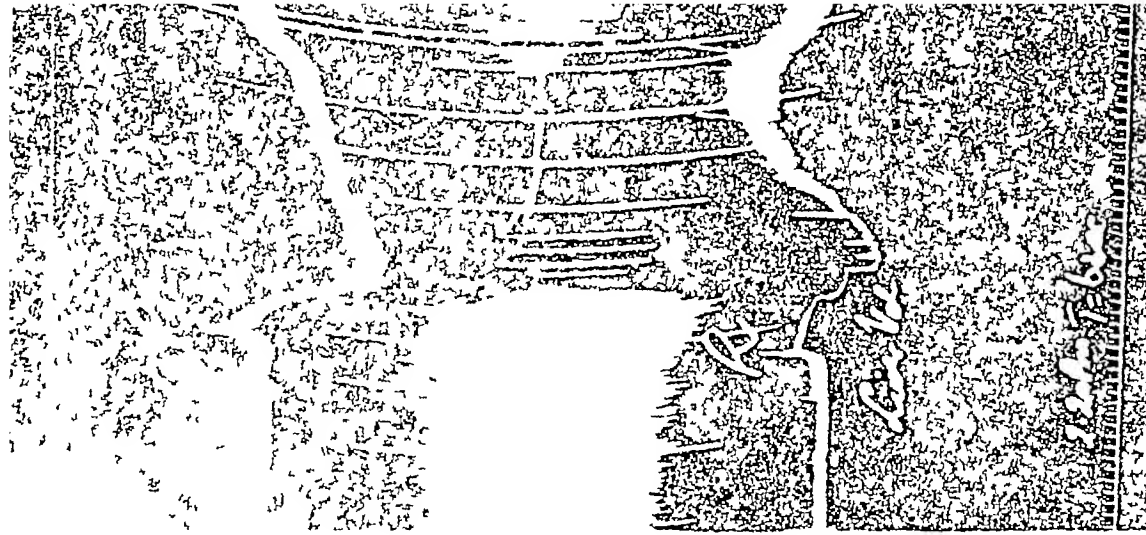
1 2 1 1  
50 m



7 4 T 6500

1 2 1 1  
50 m





## ACTION ON RESPIRATION

It is evident from Graphs IV, V and VI that small doses of antimony compounds produce little or no effect on the respiration while large doses such as 100 mg produce a well marked effect. The respirations become only momentarily stimulated when 100 mg of tartrates are given [Graph IV, fig (a)], but when similar doses of organic compounds are injected the effect is very remarkable. The respirations stop immediately with the fall in blood pressure, then become irregular, shallow and jerky and the breathing is transformed from the abdominal type to a form in which the inter-costal muscles are chiefly concerned and finally becomes altogether thoracic. The total stoppage of all movements shows that possibly the bronchial muscles are in a state of extreme tonic contraction. The respiratory changes appear to be due to three factors (a) The depression of the respiratory centre from a fall of blood pressure, (b) rise in the pulmonary pressure, and (c) contraction of the bronchial muscles. If very large doses are given both the heart and respirations stop simultaneously, this effect is more marked when the drug is injected rapidly.

## DISCUSSION

It has been pointed out that the pharmacological action of the organic compounds of antimony closely resembles that of the organic arsenicals. The most important feature of the acute symptoms of poisoning following intravenous injections of arsphenamine solutions in dogs is the marked and prolonged rise of pulmonary pressure. We have observed a similar phenomenon in cats after injections of the organic derivatives (aromatic) of antimony in use. This in itself would account for a part, if not for all the milder toxic symptoms, such as faintness, feeling of constriction in the chest, circulatory disturbances, etc., produced after injections. Cough, which is a common feature after injections of the antimony tartrates, occurs very rarely after injections of organic antimony compounds. This cough is said to be due to the precipitation of antimony oxide in the fine capillaries of the lungs and usually starts immediately after the injection and ends in vomiting. Severe toxic symptoms, such as dyspnoea, cyanosis, marked respiratory disturbances and collapse are much more common after injections of the organic antimony compounds when doses larger than the patient can tolerate have been given. This is amply borne out by our experiments on animals. The phenomenon closely resembles the 'nitritoid crises' produced after injections of aromatic organic arsenicals and apart from changes in the lung vessels, there remains the possibility that it may be associated with severe constriction of the bronchi. These lung effects in the case of arsphenamine have been said to be due to four factors (a) the effect of the drug on the pulmonary arterioles producing contraction, (b) the introduction of a large amount of alkaline solution which directly irritates the endocardium, (c) the constriction of the bronchi, and (d) pulmonary vascular obstruction caused by extensive precipitation of the drug in the fine pulmonary vessels.

As regards (a) and (b), these points have been discussed by Jackson and Smith (1918) and later by Smith (1920) and Jackson and Rapp (1920). We have shown that all the antimony compounds are acid in reaction but this is not an

important factor in producing a fall in the systemic blood pressure. As regards (c) it is seen from our tracings that after a preliminary stoppage of movement there is well marked spasmodic constriction of the bronchi.

The vascular obstruction caused by extensive precipitation in the lungs of antimonial compounds is now under investigation. This view has been supported by the fact that if the same compounds are injected into one of the portal veins, the effects ordinarily produced do not occur and it is thought that precipitation now occurs in the liver capillaries and not in the lungs.

The rise of pulmonary blood pressure and other phenomena described occur in man to a greater or lesser extent, according to dosage and idiosyncrasy as shown by the clinical symptoms. Dr J. C. Gupta noted in one of his cases that severe hæmoptysis followed an injection of 10 c.c. of a 2 per cent solution of sodium antimony tartrate.

*Liver and spleen*—It seems probable that the dilatation of these two organs after injections of solutions of antimony compounds is an important factor in the curative effects of these compounds. It has been recently shown that the spleen acts as a coarse filter to remove micro-organisms from the blood stream. Those which it cannot destroy it sends to the liver to be dealt with there. It is also well known that the spleen often becomes a habitat for bacteria and protozoa which it is unable to destroy completely or send to the liver and consequently it increases to a large size in infections such as malaria, leishmaniasis, etc. The influx of a large amount of blood charged with antimony into these organs probably helps in the destruction of the organisms lying therein. The tartrates produce these changes to a lesser degree and possibly this accounts for their lower curative effect. That engorgement with blood in these organs does occur in man can be demonstrated by the fact that after injection of antimonials most of the patients complain of a feeling of discomfort and fullness in the region of the spleen and liver. Recent work has shown that as much as 20 per cent of blood volume in the dog can be temporarily stored in the spleen.

Beside the destruction of protozoa and bacteria the detoxication of chemical substances is one of the most important functions of the liver. It is well known that in very acute toxic conditions whether chemical or bacterial, the liver may undergo acute fatty degeneration in 24 to 48 hours. Toxic substances reaching the liver through the portal circulation may produce portal cirrhosis, and toxic substances reaching through the bile channels produce jaundice and enlargement. This has an important practical bearing as marked symptoms of liver disturbances frequently occur, after injections of antimony compounds. In fact, in a majority of the cases who have taken a course of injections of these compounds a mild type of jaundice 2 to 3 weeks after the course is quite a common occurrence. The effect of the antimonials on the liver, however, is not so severe as those of the arseno-benzol compounds.

#### SUMMARY AND CONCLUSIONS

1. All the organic compounds of antimony produce a fall in the systemic blood pressure after intravenous injections. This fall is easily compensated for when small doses are injected but if large doses are given and specially if given



rapidly, the fall is pronounced and lasting. The blood pressure soon regains its normal level unless very large doses have been injected.

2 The fall in the systemic pressure is due partly to the lessened output of the left ventricle and partly to dilatation of the splanchnic vessels. The acidity of the solutions is not an important factor in producing the fall.

3 The cardiometer shows that the heart is considerably dilated and both the force and the amplitude of the contractions are decreased. This weakening of the beats is also shown by the myocardiograph and in records of the contractions of the isolated heart.

4 The pressure in the pulmonary artery and also in the inferior vena cava rises. With small doses, given slowly, the rise is small and is immediately compensated for. With larger doses, given rapidly, the pulmonary pressure increases 100 per cent or more and persists at that level for some time. This rise is due to progressively increasing obstruction in the lungs which causes the right heart at first to do more work and then to dilate when the strain is beyond its power. At this time the systemic pressure has fallen and the venous pressure rises.

5 The blood vessels of the intestines, liver and spleen dilate, the kidney shows slight contraction, and the limb also slightly decreases in volume.

6 The increase in the size of the spleen and liver after injections is very remarkable, the rhythmic movements of the spleen are increased. The influx of a large amount of blood into these organs is probably an important factor in the curative effect of these compounds.

7 The respiration is only slightly stimulated even with large doses of the tartrates but with large doses of the organic compounds it stops immediately with the fall in blood pressure. Respiration becomes irregular, shallow and jerky and changes from the abdominal type to a thoracic type. When large doses are rapidly given both the heart and respiration may stop simultaneously.

8 The circulatory changes in the pulmonary, systemic and venous systems and the changes in the circulation of the spleen, liver, etc., account for all the milder toxic symptoms such as faintness, feeling of constriction in the chest, cough, feeling of fulness in the region of spleen and liver, etc. The severe toxic symptoms such as dyspnoea, cyanosis, marked respiratory disturbances resembling 'nitroid crises' are associated with extreme changes in the pulmonary pressure as well as contraction of the bronchi.

I am very grateful to Dr. L. E. Napier for his advice and for supplying me with specimens of different organic antimonials and to Drs. J. C. Gupta, J. C. David and K. Venkatachalam for their help in the experimental part of this research.

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PSORALIA CORYLIFOLIA (BABCHI) ITS CONSTITUENTS,  
THEIR PHARMACOLOGICAL ACTION AND  
THERAPEUTIC PROPERTIES

BY

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*Psoralia corylifolia* Linn, is a common herbaceous weed belonging to the natural order leguminosæ which grows throughout the whole length and breadth of the plains of India. It is called *Bukchi* or *Babchi* in Hindi, *Latakasturi* or *Bavachi* in Bengali and *Sugundha Kantik*, and *Vakuchi* in Sanskrit. The seeds of this plant have been in use in Hindu medicine for a long time. They are brownish black in colour and are about 2 mm long and are oblong and flattened. They are hard but not brittle, have a soft skin, an agreeable aromatic odour and a pungent bitterish taste. No oil can be expressed from the seeds even under high pressure. A very good quality of *bukchi* plant grows in Rajputana and the seeds can be bought in the market at Rs 15 to Rs 20 per maund.

The seeds have been described by the ancient Hindu physicians as 'hot and dry' and according to some 'cold and dry, lentive, fragrant, stimulant and aphrodisiac'. They have been specially recommended in leprosy internally and are also applied in the form of paste or ointment externally. The drug has been considered to be so efficacious in this disease that it was given the name of *Kushtanasini* (leprosy destroyer). In inflammatory diseases of the skin, leucoderma and psoriasis it is given both as a local application and by the mouth.

The seeds are also used as an anthelmintic, diuretic and diaphoretic in febrile conditions. Several species of *Psoralea* grow in America and are used medicinally in that country as a stimulant and as a nervous tonic.

### CHEMISTRY

Dymock in his 'Pharmacographia Indica' states that *babchi* seeds contain a colourless oil, extractive matter 13.5 per cent, albumin, sugar, ash 7.5 per cent and a trace of manganese. Very little work was done on this drug until recently, when Sen, Chatterjee and Datta made a thorough examination of the seeds. These authors found that the seeds contain (1) an unsaponifiable oil having the formula  $C_{17}H_{21}O$  boiling between  $180^{\circ}$  and  $190^{\circ}C$  at 11 to 15 mm, (2) a yellow acid substance  $C_{40}H_{15}O_{10}$  from alcoholic extract, (3) a methyl glucoside having a melting point of  $105^{\circ}$  to  $107^{\circ}C$  containing four (OH) groups. They found the unsaponified oil to be pharmacologically active and they used it with success in cases of leucoderma and psoriasis. They did not, however, study the essential oil present in the seeds, which was associated with their unsaponifiable oil. Our investigations are chiefly on the essential oil present in the seeds and we found it to be mainly responsible for the pharmacological and therapeutic action of the drug.

#### Preliminary Examination —

- (a) Microsublimation—no definite sublimate was obtained
- (b) Extraction with warm water—presence of tannins, gums, sugar, poly-saccharide or glucoside was indicated, by Fehling's solution
- (c) Extraction with cold acidulated water—faint alkaloidal reaction was obtained
- (d) Extraction with solvents—25 grams of powdered seeds (No. 60-powder) were extracted successively with the following solvents —

	Grams	Per cent.
Petroleum ether (B.P. $50^{\circ}$ )	4.14	16.56
Ether	2.14	8.56
Chloroform	0.54	2.16
Ethyl acetate	0.22	0.88
Alcohol	0.61	2.44

#### Systematic Examination —

##### I Extraction with petroleum ether—

One kilogram of powdered seeds was repeatedly extracted with petroleum ether (B.P.  $50^{\circ}$ ) until exhausted. The extract was concentrated to a small bulk and kept in a cold place for a week, when a crystalline deposit was obtained. The mother liquor was decanted off and the crystals were washed with petroleum ether, in which they were sparingly soluble, they were found to be needle shaped. The substance was of bitter taste, insoluble in water. It was slightly soluble in ammonium carbonate, fairly soluble in hot sodium carbonate solution and soluble in hot 10 per cent caustic soda solution, from which most of it was precipitated on acidifying. It was fairly soluble in ether and very soluble in absolute alcohol. The crystals obtained from ether melt at about  $135^{\circ}C$ . The substance was probably a resin acid.

The mother liquor after separation of the crystals was diluted with twice its bulk of petroleum ether and repeatedly shaken with 10 per cent sulphuric acid. The acid extract was made alkaline with sodium carbonate and shaken with petroleum ether. The solvent was evaporated and the residue was taken up by dilute hydrochloric acid. It gave a precipitate

with Meyer's solution and other alkaloidal reagents, indicating that traces of an alkaloid may be present. The acid extract does not reduce Fehling's solution both hydrolysed and unhydrolysed.

The petrol ether extract was then washed with water and dried with anhydrous sodium sulphate, filtered and the solvent evaporated. To it 90 per cent alcohol was added and shaken well, when the liquid stood in two layers. This process was repeated thrice when the alcoholic extracts were all collected together, evaporated to dryness, and then distilled in steam. An essential oil having the distinct smell of the seeds distilled over, this was collected and identified separately, as described below.

The residue after steam distillation was a red coloured oily substance which was composed mainly of resins.

The dark coloured liquid obtained after separation with 90 per cent alcohol, being mostly fat and fatty oil, was saponified with alcoholic potash. When the saponification was complete it was poured into four times its volume of water and the mixture was repeatedly shaken with ether. The ethereal solution was washed with water, dried and the solvent evaporated. The unsaponified product was a brown oil having a sweet odour. 10.723 gm of the oil gave 0.393 gm unsaponified product.

The soap solution was then acidified with dilute hydrochloric acid, when the fatty acids separated. It was washed with hot water, on cooling to ordinary temperature it solidified. The fatty acids were not further studied.

II The residue after extraction with petro-ether was freed from the solvent and was repeatedly extracted with ether.

A concentrated solution of the ethereal extract does not precipitate anything on keeping undisturbed for about a week. It was tested in small portions as follows—

- (a) Shaken with 1 per cent sulphuric acid, the acid extract was tested with Meyer's reagent, giving no alkaloidal reaction.
- (b) Shaken with water and the watery solution was tested with (i)  $\text{FeCl}_3$ —giving bluish green colour of tannins. (ii) Naphthol and  $\text{H}_2\text{SO}_4$ —gave no carbohydrate reaction. (iii) Fehling's solution—was not reduced by both hydrolysed and unhydrolysed solution.
- (c) Treated with 90 per cent alcohol, but no separation was effected. On diluting the alcohol almost the whole of the substance was precipitated.
- (d) A small portion was shaken with KOH solution without any definite separation.

As no other bodies could be separated, the whole of the extract after driving off the solvent was washed with water, dried and weighed as resin. It was a deep coloured oily substance and did not solidify on standing. Its weight was 91 gm. It was purified by dissolving in alcohol and pouring into a large bulk of water and then dried. 20 per cent solution of the purified resin was supplied to the out-patients department for skin diseases and was found to have no action.

III The residue after extraction with ether, having been freed from the solvent, was exhaustively extracted with absolute alcohol. On concentration and keeping, nothing separated from the alcoholic extract.

On distilling off the solvent from the extract, it was shaken repeatedly with water. The watery extract was tested for alkaloid and was found to be negative, it reduced Fehling's solution on hydrolysis while it slightly reduced unhydrolysed solution.

The whole of the watery extract was precipitated with neutral lead acetate. The precipitate was suspended in water and decomposed by  $\text{H}_2\text{S}$ . The filtrate was tested with ferric chloride, when a deep blue precipitate was obtained, indicating the presence of tannins. The filtrate was precipitated with basic lead acetate, when a yellow precipitate was obtained. It was washed with water, dried, suspended in alcohol and decomposed by  $\text{H}_2\text{S}$ . The residue on evaporation was a small amount of yellow semi-solid body, which did not reduce Fehling's solution, but reduced the same on hydrolysis with acid. It was probably the glucoside, obtained by Sen, Chatterjee and Datta, previously described.

No definite body could be isolated from the filtrate from basic lead acetate

The alcoholic residue after extraction with water solidified to a reddish brown powder. It was soluble in alkalis and was precipitated by acids. It was mainly phlobaphenes, weighing 29 grams

IV The residue after extraction with alcohol was extracted with water. From the watery extract, tannins were separated by neutral lead acetate and the filtrate was precipitated by basic lead acetate. The precipitate on decomposition by  $H_2S$ , was found to be the same product, of glucoside nature, as that obtained from the alcoholic extract.

#### *Examination of the essential oil —*

As the quantity of essential oil obtained by the above preliminary examination was very small, 3 kilos of crushed seeds were distilled in steam and the distillate collected. The distillate was saturated with common salt, when most of the oil floated at the top, and repeatedly extracted with ether. The ethereal extracts were collected and dried with anhydrous sodium sulphate. On slowly evaporating the solvent a straw-coloured essential oil having the characteristic odour of the seeds was obtained, the yield being 0.05 per cent.

*Density of the oil* —The specific gravity of the essential oil was determined by the pycnometer and was found to be 0.9072, at 25°C.

*Refractive index* —The index of refraction of the essential oil was determined, by an Abbe refractometer at 25°C and was found to be 1.5025.

*Solubility* —The solubility of the essential oil was determined by adding 0.079 gm oil in 400 c.c. water, shaking it from time to time and allowing the mixture to stand overnight. Next day, it was cooled to 25°C and the clear liquid was drawn out, filtered and collected up to 250 c.c. It was shaken repeatedly with ether. The ethereal extract was taken into a tared glass basin, the solvent evaporated and the residue was dried in a desiccator. The solubility was found to be 0.0197 per cent or about 1 in 5,000.

*Optical rotation* —A 2 per cent solution of the oil in chloroform showed no angular rotation in a Laurent polarimeter.

The essential oil when stored in a sealed tube kept for a considerable period, the colour, however, gradually turned to deep brown, but when placed in a desiccator over calcium chloride or exposed to air, it crystallised in needles, probably due to oxidation of some of its constituents. The crystals had a sharp cooling taste. It melted sharply at 126°C. If the temperature was further raised to 330°C, it slowly turned black, showing the decomposition of the substance at a high temperature, without boiling at ordinary pressure. On cooling it was found that a well-defined needle-shaped crystalline sublimate had deposited on the cooler parts. As the quantity of the essential oil at our disposal was very small, it could not be fractionated *in vacuo* to study its constituents.

*Preparation of the Oleo-resinous extract* —1 lb of the powdered seed was thoroughly mixed with 1 lb of olive oil and the mixture was kept overnight. Next day, it was transferred into a tincture press and the oil expressed. About half a pound of oil was collected and filtered through cotton-wool. The oil might be diluted with fresh olive oil according to requirements.

### THE PHARMACOLOGICAL ACTION OF THE ESSENTIAL OIL

*Externally* The oil has an irritant effect on the skin and mucous membrane. Its action on undifferentiated protoplasm such as paramœcium is quite marked. In 1 in 50,000 dilutions of the essential oil, the paramœcia remain alive and active for 15 minutes, after 25 minutes movements were somewhat slowed and some died in 40 to 45 minutes. In 1 in 10,000 concentration these organisms are killed in 10 minutes. The essential oil shows a selective activity against the skin streptococci and that in all probability accounts for its extensive use by the Hindu physicians in skin affections. The following table gives the relative effects of

1 0 per cent phenol and different dilutions of the *babchi* essential oil on the skin streptococci —

	Time in minutes			
	2½	5	7½	10
Phenol, 1 0 per cent	—	—	—	—
Saturated solution of essential oil	+	—	—	—
Dilution 1 in 10,000	+	+	+	—
„ 1 in 25,000	+	+	+	+
„ 1 in 50,000	+	+	+	+

Saturated watery solution of the essential oil of *babchi*, solubility 1 in 5,000 —no growth, + growth

Dilutions of 1 in 10,000 kill skin streptococci in 10 minutes. Against *B. typhosus* (Calcutta strain) the essential oil has no activity at all and there was growth of these bacilli in all concentrations. We also tried the effect of the essential oil on the cholera vibrio and *B. dysenteriae* (Flexner) with results similar to those obtained with *B. typhosus*.

On voluntary muscle, the essential oil in high dilutions (1 in 50,000 to 100,000) has a distinct stimulant action. The tone of the isolated uterus of the guinea-pig or cat is decidedly increased and the uterus may show a tonic contraction. Perfused, isolated pieces of intestine are similarly affected and the peristaltic movements are increased. Saturated solutions of the oil injected intravenously have no effect on the blood pressure. The isolated mammalian heart shows neither stimulation nor depression. On perfusion with 1 in 5,000 solution of the oil there is well marked contraction of the arterioles in a frog. The respiration is not affected.

#### THERAPEUTIC USES OF *Psoralea corylifolia*

*Psoralea corylifolia* seeds are a very ancient remedy for leucoderma, they have been tried extensively not only by the practitioners of the old Hindu medicine but by members of our own profession. Kanny Lall Dey strongly recommended an oleo-resinous extract and he describes the effects as follows —

‘After application for some days the white patches appear to become red or vascular, sometimes a slightly painful sensation is felt. Occasionally small vesicles or pimples appear and if these be allowed to remain undisturbed, they dry up, leaving a dark spot of pigmentary matter, which forms as it were a nucleus. From this point as well as from the margin of the patch, pigmentary matters gradually develop, which ultimately coalesce with each other, and thus the whole patch disappears. It is also remarkable that the appearance of fresh patches is arrested by its application.’

Other observers have not obtained such good results

Lieut-Col H W Acton, I M S, in-charge of the Skin Outpatient Department, kindly tested a number of preparations made from *Psoralea corylifolia* seeds in various skin affections 1 in 10,000 to 1 in 20,000 solutions of the pure essential oil was tried in some cases of acute streptococcal dermatitis but unfortunately it set up much irritation and made the condition worse A 20 per cent solution of the purified resin in alcohol was quite ineffective in leucoderma A 10 per cent solution of the essential oil in alcohol was also unsatisfactory The oleoginous extract made from the seeds already described was the most suitable preparation, this contains most of the essential oil present in the seeds This oil was applied locally to leucodermic patches by gentle rubbing once or twice daily Cases of leucoderma in the Skin Department of the Calcutta School of Tropical Medicine are divided into two groups—

I *The primary group*—i.e., those not associated with any other skin disease These are sub-divided into those of syphilitic origin and those of non-specific origin These are further divided into those showing *E histolytica* and other affections of the gastro-intestinal tract and those which are free from it

II *The secondary group*—includes cases which are associated with other diseases of the skin such as ringworm, seborrhœic dermatitis, etc The oleo-resinous extract was tried in 600 cases of leucoderma of both groups but its beneficial effects were noticed only in the non-syphilitic group In the syphilitic cases it had no effect, because here in all probability the melanoblasts are killed, as they are not visible in the histological preparations

The effect of the essential oil is purely local The Hindu physicians give the powdered *babchi* seeds by the mouth, but we have not tried these in leucoderma The beneficial effects may be due to—(1) absorption and excretion of the oil through the skin when it produces its specific action, (2) the stimulant action on the intestinal mucosa which may cause increased absorption of amino-acids concerned in pigment formation, or (3) antiseptic action in the gastro-intestinal tract, but this is not borne out by our experiments Our observations show that the effect of the essential oil is purely local and therefore any existing concurrent affections of the gut such as infection with *E histolytica* should be treated at the same time The action of the oil on the skin appears to be specific Krogh has demonstrated that Rouget's cells lie round the capillaries The endothelium of the capillaries by itself has no contractile power and any increase or diminution in the size of these vessels is brought about through the agency of the processes of Rouget's cells In the skin the melanoblasts or pigment producing cells lie in the vicinity of Rouget's cells When the capillaries dilate Rouget's cells also increase in size and the melanoblasts relax at the same time During relaxation of the melanoblasts their processes are extended and they exude the pigment melanin The main action of the essential oil appears to be on the arterioles in the sub-capillary plexuses causing dilatation and increase of plasma in this area so that the skin becomes red and the melanoblasts are stimulated The action on the capillaries in the papillæ is usually very slight in most individuals so that there is no œdema of the prickle cells layer (poro-keratosis) and there is no desquamation of the epithelium

The essential oil, however, varies enormously in its effects on different persons. With the majority (95 per cent) it causes only redness of the leucodermic patches but in a small number (5 per cent) there is extreme sensitiveness to the oil, so much so that blistering may be produced. This indicates that not only is dilatation of the blood vessels produced but at the same time the permeability of the capillary tufts is markedly increased so that fluid accumulates and blisters form between the prickle cells and the capillary layer of the skin. In yet another class of cases blistering only occurs after the application of the oil if the skin is exposed to the direct rays of the sun. The strength of the oil should therefore be varied in such a way as not to allow its action to go beyond the state of redness of the leucodermic patches. The oil being an essential oil, and therefore volatile, is able to permeate through the epidermis to the prickle cells of the lymphatics and so find its way to the sub-capillary area and stimulate the cells situated there. The advantage of this oil over the other skin irritants (compounds of mercury, salicylic acid, etc.), is that it does not produce desquamation or any change of keratolytic nature resulting in loss of pigment of the epidermis. So far as is known *babchi* is the only drug that has a dual action, i.e., action on both Rouget's cells and the melanoblastic cells of the skin. This specific action of the oil can be readily demonstrated on the frog's skin under a microscope. In leucoderma the melanoblastic cells are not functioning properly and their stimulation by the oil leads them to form and exude pigment which gradually diffuses into the decolorised areas.

#### SUMMARY AND CONCLUSIONS

(1) The active principle of the seeds of *Psoralea corylifolia* (*babchi*) is an essential oil. A fixed oil and a resin occur in large quantities but these are not pharmacologically active substances. Traces of a substance of alkaloidal nature are also present.

(2) The essential oil has a powerful effect against the skin streptococci. It has a specific effect on the arterioles of the sub-capillary plexuses, these it dilates so that in this area plasma is increased. The skin becomes red, the melanoblasts are stimulated leading to pigment formation. This pigment is exuded and diffuses into the decolorised leucodermic patches.

(3) Local applications of the oleo-resinous extracts made from the seeds are beneficial in the treatment of cases of leucoderma of non-syphilitic origin. If affections of the gastro-intestinal tract such as *E. histolytica* infections, etc., are present, these should be treated at the same time.

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## OPIMUM HABIT IN INDIA

### AN ANALYSIS OF 100 CASES AMONGST THE SIKH POPULATION OF CALCUTTA

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THE commonest drug addictions in India are alcohol, opium, cannabis indica and cocaine. The opium habit is the oldest of all the drug habits in this country, it is very prevalent in certain parts, and among certain classes of the population. Opium, the inspissated juice of the poppy, appears to have been introduced into India by the Mohammedan invaders, and it was Emperor Akbar who first made a State monopoly of the opium trade. It is difficult to say to what extent opium addiction was prevalent in the time of the Moghuls, but it is said that at one time most of the nobility were indulging freely in the habit. A large amount of opium was produced and some must have been exported. It is said that when the Portuguese first entered the China seas in the 16th century, they found opium an important article of trade carried on by Indian and Arab merchants. During the 17th century, Dutch, English, French and Danish merchants purchased large quantities of opium in India from the Moghul Government for export. After the fall of the Moghul Empire, the State lost its hold on the monopoly, and the control of the production and sale of opium was appropriated by a ring of Patna-merchants. When the East India Company assumed the responsibility for the collection of the revenue in Bengal and Behar, some of its officers took the control of the opium into their own hands and monopolised

its trade for their private benefit. When Warren Hastings was appointed Governor-General, he was authorised to organise a proper system of Government and he brought the whole monopoly of opium trade under the control of the Government. Since then, though changes have been made in the method of control, in production, distribution, sale and possession of opium, the monopoly has been solely in the hands of the Government.

There has always existed in other countries opposition against the Indian opium trade. Owing to the activities in England of the Society for the Suppression of the Opium Trade, a Royal Commission was appointed in 1893 to inquire into all the circumstances connected with the production and sale of Indian opium. The labours of this Commission resulted in the publication of its report in seven volumes. After considering the question of consumption of opium in India, the Commission came to the conclusion that opium was a common domestic medicine and afforded protection and cure against malaria and other fevers. The habit scarcely existed as a vice, for centuries of inherited experience had taught the Indian discretion in the use of this drug, and the great majority of the opium eaters in India were not slaves to the habit, for they could give up the use of it when it was no longer necessary. The Commission was of opinion that the habit had no deleterious effect either morally or physically, for hard work, energy and thrift existed side by side with the eating of opium, that the habit was generally indulged in after the age of 40 and it was often considered a boon in old age.

The fear of the spread of the habit from India and China to other parts of the world had aroused an international interest in opium and this eventually resulted in the institution of an International Opium Commission in 1909 at Shanghai, and the present policy of restriction of the Government of India dates back from the findings of this Commission. The Government of India accepted cordially the principles laid down by this Commission, namely, the necessity for the gradual suppression of opium smoking and the advisability of either prohibiting or regulating carefully the use of opium for any other purpose than medicinal. It is beyond the scope of this paper to go into the various international discussions that took place at the Hague Convention of 1912 or the 1st and the 2nd Opium Conferences at Geneva in 1924 about the effects of addiction to all narcotic drugs. It will suffice here to say that as a result of these international conventions and conferences, considerable interest has been aroused in India and other countries on this question and various contradictory views have been expressed from time to time.

In a discussion at the Royal Society of Medicine, London (1924), on drug addiction in the tropics, some authorities with considerable Indian experience thought that the opium habit did no harm but that it conferred certain beneficial effects on those who took it in small quantities. It was urged by others that the drug was considerably abused in India, and was doing a great deal of harm. The official opinion was also divided on this subject. On the one hand the ill effects of this habit were considered to be not immediate but to lead to dullness, want of enterprise, carelessness of appearance, and general weakness of character. The addicts became extravagant and fond of gambling and got into debt. The spread of the habit was having a lowering effect on the general morality of the people.

On the other hand it has been stated that as a vice it scarcely exists in India as it is only taken in moderation by the average Indian. Opium is only eaten as a mild stimulant, as a prophylactic against malaria, or for the relief of pain in various diseases. It is, in fact, a household remedy for all painful diseases and is prescribed by them after centuries of experience.

Most of the views about the harmlessness of the opium habit are based on the findings of the Royal Commission of 1895. No inquiry on scientific lines has been instituted since then though conditions have changed considerably, and medical aid has been increased. Recently the National Christian Council and the Indian National Congress have made inquiries chiefly from social and economic considerations and have shown that the habit is very prevalent in certain parts of India and is doing considerable harm. The League of Nations laid down that the legitimate consumption of opium in a country which has a developed medical service should not exceed 6 seers or 12 pounds per 10,000 of the population per annum. It has been shown that the average for the whole of India is 12 seers, i.e., double the standard laid down by the League. It is, however, said that there is no such universal extension of opium habit in India as to cause alarm. The reason of the higher consumption is that there is a most startling disparity in the amount consumed in different parts of the country. In the Punjab, for instance, though the consumption rate per 10,000 is 11.9 seers it is found that in some districts of that province (e.g., Ferozepur, Ludhiana, Lahore, etc.), the consumption varies from 40 to 60 seers while in others (e.g., Jhelum), it is less than 3 seers. In Bengal the average is 8 seers, but in Calcutta it is 143.6 seers, in Assam it is 52 seers, in Behar and Orissa 8 seers. These figures do not include the opium which is undoubtedly smuggled into these areas.

These figures show that there is abuse of opium in certain parts of the country and among certain classes. It cannot be acceded that opium is necessary as a household medicine.

The National Christian Council strongly supported the plea of the League of Nations for restricting the use of opium to purely medical and scientific needs, i.e., roughly 15 grams per head per annum or 6 seers per 10,000 of population per annum. During the discussion in the Legislative Assembly last year it was shown that in many districts in certain provinces where efficient medical aid was available there was a high consumption of opium. It will, therefore, be seen that there is a great deal of difference of opinion on this point and an inquiry on scientific lines at this stage would be very advantageous to collect all the available facts. We are greatly indebted to the Governing Body of the Indian Research Fund Association for placing funds at our disposal to carry out this inquiry.

The inquiry we are conducting consists of —

1. Collection of statistics from all the available sources about the production and sale of opium and determining by work in the field, the extent to which this drug habit is prevalent among the population.

2. Collection of a large number of cases of addiction with a view to determine the causes leading to the habit, the quantity ingested and the physical, mental and moral effects produced by it.

3 Inquiry into the pathological changes produced by examination of post-mortem records and by experiments on animals A perusal of the literature shows that little work has been done in this direction

4 The treatment and the cure of the habit

As the inquiry is a very difficult and extensive one we have started work on class lines and have first taken up the study of the opium habit in the Sikh community, as it is very prevalent amongst them We have further sub-divided the Sikhs into those following vocations bringing in good income, e.g., taxi-drivers in Calcutta, those living in rural areas, those serving in the army, and those belonging to the higher and middle classes This paper embodies the results of our work among the Sikh population of Calcutta We have collected a series of one hundred cases among this community and have carefully gathered and recorded all available information concerning the family history, personal history of the addict and the age at which the habit was started We went into the causes which lead to the formation of the habit, the dose with which the start was made, any increase in the dosage, the duration of the habit, the symptoms produced after taking the drugs and the symptoms of abstinence We further inquired whether the habit conferred any protection against any disease and obtained information about the immediate and remote physical, mental and moral effects that might be produced by it and the addict's own verdict on his habit, that is, whether he considered it beneficial or otherwise This could only be done by gaining the confidence of the persons concerned and this we were fortunate enough to do through the kindness of one of the popular Sikh practitioners of Calcutta whose practice mainly lies among that community He obtained for us the names of the persons who indulged in the habit in the Sikh community, and enabled us to get in touch with them It may be stated here that the majority of the Sikhs in Calcutta are engaged in taxi driving, or as 'durwans' or watchmen or as artisans Our conclusions therefore mainly apply to these classes

Before analysing our data it will not be out of place to say a few words as to how opium is taken in this country It is generally eaten in the form of pills and in some parts it is dissolved in water or a decoction is made with tea In some districts in the Punjab an aqueous extract of poppy capsules is used The capsules are soaked overnight and in the morning are mashed with hand into a fine pulp so as to dissolve the alkaloids present, the fluid part is separated from the mash and is drunk Others make a tea-like decoction from the poppy capsules The capsules used for this purpose are those from which opium has not been extracted but even so the amount of alkaloids present is very small and the effects produced are very mild in character

Opium smoking, which is the common form of consumption in other Eastern countries, is said to be very uncommon in India That it does exist among certain classes is evident from the fact that the word 'Chandubaz'—opium smoker—is well-known and in some of the Hindusthani books very detailed description has been given of this habit In most parts of India this is a very uncommon method of consumption but investigations of the National Christian Council and the Indian National Congress show that in Assam opium smoking is almost as common as opium eating and it is estimated that from one-third to

half of the addicts in that province smoke this drug We will not enter into discussion as to which method is more injurious, opium smoking or opium eating Much has been said on either side but no proper inquiry has been made and we hope to take it up later In the present series of cases the drug was eaten

*Causation*—A perusal of Table A I shows that 58 per cent of the habitués acquired the habit from association with other habitués, and 30 per cent from the use of the drug for some disease or ailment for which no medical advice was sought by them Only 4 per cent of these started taking it under the advice of a physician so that medical dispensing does not play any appreciable part in inducing the habit in India as it appears to do in European countries Fatigue, worry dissipation and old age play a minor part in this series It is also clear from the table that when the habit is acquired by association, the daily amount consumed increases rapidly, the majority taking over 10 grains a day When the habit is formed from other causes, e g, pain, etc, the addict, as a rule, remains satisfied with a smaller dose

Two cases in this series are worthy of note as being peculiar and of special interest One man was partaking of opiated tea for a long time with a friend of his without knowing anything about it This friend went away for a day and although he took the ordinary tea he felt miserable He soon discovered the reason and since that time he has continued taking opium and has not been able to get rid of the habit The other is the case of a demobilised sepoy who, while on active service during the great war, was entrusted with a quantity of opium to be taken to a friend at the front He was unable to accomplish his mission and instead of throwing away the opium he thought he might try it on himself and has taken it ever since

*Occupation*—It will be seen from Table A II that the addicts follow different vocations The majority are engaged in driving motor cars, especially taxi cars, which ply for hire These individuals have not only long hours of work, but are exposed to all sorts of climatic conditions They get plenty of leisure in the intervals between fares, they congregate together and they are very well off financially for that class of individuals All these facts probably account for their indulging in the habit to a greater extent, 54 per cent of our cases were motor and taxi drivers The next biggest number, i e, 23 per cent, is amongst the 'durwans' or watchmen who lead an uneventful life of dullness and monotony Among the artisan and other classes, the habit appears to be comparatively less prevalent It will also be noted in this connection that the motor drivers on the whole have a tendency to consume larger quantities, the majority of them taking over 10 grains a day

*Duration*—A perusal of Table A III will give an idea about the duration of the opium habit among this series In 14 per cent of our cases the habit continued for under one year, in 29 per cent for 1 to 5 years, in 22 per cent for 6 to 10 years, and in 35 per cent for over 10 years This table brings out the interesting fact that in 57 per cent of the cases the habit once acquired continued for long periods, and one is justified in inferring that once the habit is established it is difficult to get rid of It will also be seen that those in whom the habit has lasted longer show a tendency to a bigger dosage.

TABLE A

Dose taken	I CAUSATION OF ADDICTION						II OCCUPATION							III DURATION							IV PRESENT AGE							Total					
	Association	Disease	Medical advice	Fatigue, worry etc.	Old age	No cause	Motor and Taxi drivers	Durwans	Shop-keepers	No work	Soldiers	Servants	Artisans	Under 1 year	Under 2 years	Under 3 years	Under 4 years	Under 5 years	From 6—10 yrs	From 11—20 yrs	From 21—30 yrs	31 yrs and over	Under 20 years	From 21—30 yrs	From 31—40 yrs	From 41—50 yrs	From 51—60 yrs		From 61—80 yrs	81 yrs and over			
From 1—5 grains	5	8	—	2	—	—	7	6	—	2	—	—	—	6	1	—	1	—	—	2	3	1	1	—	1	6	6	—	2	—	15		
From 6—10 grains	15	8	1	1	1	—	15	9	1	—	—	—	1	3	2	4	4	2	5	3	3	—	—	8	5	8	4	1	—	—	26		
From 11—20 grains	22	8	—	2	—	—	17	7	1	3	—	3	3	3	3	4	1	3	7	6	6	1	2	13	9	5	3	1	1	—	34		
From 21 grains and over	14	6	3	1	—	1	15	1	3	2	1	2	1	2	1	1	—	2	8	7	3	1	—	8	12	5	—	—	—	—	25		
	58	30	4	6	1	1	54	23	5	7	1	5	5	14	7	9	6	7	22	19	13	3	2	30	32	24	7	4	1	—	100		
	100						100							100							100							100					

TABLE B  
*Age of commencement of the habit*

Dose taken	20 years and under	From 21—25 years	From 26—30 years	From 31—35 years	From 36—40 years	From 41—45 years	From 46—50 years	From 51—55 years	From 56—60 years	61 years and over	TOTAL
From 1 to 5 grains	—	—	5	3	2	2	3	—	—	—	15
From 6 to 10 grains	4	7	1	5	2	1	1	3	—	—	27
From 11 to 20 grains	6	12	6	4	—	1	2	—	—	1	32
21 grains and over	11	8	1	2	—	1	—	—	—	—	26
	21	27	19	14	4	5	6	3	—	1	100

*Age*—A perusal of Table A IV shows that only 2 per cent of the addicts were under 20 years of age, 86 per cent were from 21 to 50 years old and 12 per cent over 50 years old

A reference to Table B reveals the interesting fact that in 81 per cent of this series the habit started before the age of 35. A detailed examination of the table shows that in 21 per cent a start was made under 20 years of age, in 46 per cent between the ages of 21 and 30 years, in 18 per cent between the ages of 31 and 40 years, in 11 per cent between the ages of 41 and 50 years and only in 4 per cent over the age of 50 years. It is obvious from both these tables that the idea that the habit is started in India in the majority of cases after the age of 40 years and which was supported by the findings of the Royal Commission of 1895 is not borne out in this series. It will also be seen here that many of the younger addicts show a tendency towards taking larger doses than those who acquire the habit in later life who remain satisfied with comparatively smaller doses.

*Heredity and Association*—Table C shows that in 41 per cent of the cases the habit existed in some of the other members of the family and in 50 per cent of these some of the older members such as grandfather, father, mother, uncle, etc., were taking opium. In 59 per cent no family history of addiction was given. It would appear that heredity does not play any part in inducing the habit. It is evident from Table A I that association with other addicts and their example is responsible for the spread of the habit to others in the same family as well as to the outsiders.

The family histories of the cases in this series record only three cases of opium habit among the women and these were in elderly ladies, being in all cases the mothers of addicts. This shows that the opium habit is very uncommon among the females.

*Quantity consumed*—A perusal of the tables shows that in this series 15 per cent took 1 to 5 grains, 26 per cent 5 to 10 grains, 34 per cent 10 to 20 grains and 25 per cent over 20 grains per diem. Of this last group quite a number



TABLE C  
Addiction in the family

Dose taken	Father	Brother	Uncle	Grandfather	Cousin	Women members of the family	Two or more members of the family	No addicts in the family	Total
From 1 to 5 grains	1	1	1	—	—	—	4	6	13
From 6 to 10 grains	3	—	1	1	—	2	4	18	29
From 11 to 20 grains	6	3	—	—	1	1	1	20	32
21 grains and over	3	1	2	1	—	—	4	15	26
	13	5	4	2	1	3	13	59	100

took over 50 grains a day. The plea of moderation in the quantity of the drug taken by the addicts generally is therefore not borne out by this series. These figures also help in fixing the average dose of the addicts which can be safely put at 15 grains per day.

*Symptoms produced* —The majority of the addicts we examined could not give any clear description of the symptoms produced. Increased activity, inclination to work, sense of well being, a feeling of pleasure and mild excitement were all that were described as the effects of indulgence in the drug. Others said that they only felt normal after they had taken a dose, there were still others who did not get any noticeable effects at all but were afraid to give it up because of the abstinence symptoms.

*Physical and mental effects* —The consensus of opinion among medical men is that physical deterioration is a factor in the toxic states but in addiction there is the psychical disturbance which constitutes the true dominant therapeutic problem. This series of cases is too small to give any idea as to the effects produced on the physical, mental and moral state of the addicts, but a careful study of the individuals shows that the habit decreases their working capacity, as the life of the addicts is dominated by the fear of abstinence symptoms which start when the effect of the drug begins to wear off and which render the individuals very uncomfortable and almost abnormal if the supply of the drug is not at hand. 65 per cent of the habitués in this series declared that the habit was harmful and they wanted to give it up but were afraid to do so because of the withdrawal symptoms. We could not get any evidence bearing on the statement sometimes made, that motor drivers addicted to opium were responsible for a greater number of accidents.

*Association of opium habit with other drugs* —52 per cent of these cases gave a history of taking alcohol in some form or other. Some of the addicts took it occasionally and others regularly. The association of the habit with 'bhāng' or *Cannabis indica* was rare and was noted in only one case in this series. Tobacco smoking was often associated with this habit and even religious scruples

were set aside. Quite a number of the Sikhs in this series, to whom tobacco is forbidden by their religion had taken to smoking.

*Symptoms of abstinence*—Symptoms of abstinence start as soon as the effects of the drug begin to wear off. The majority of our cases in this series took the drug once in 24 hours but quite a large number took it twice a day. Yawning, sneezing, lacrimation and hoarseness were among the early symptoms when the drug was withheld. Later restlessness, nervousness, pains in the limbs, insomnia, nausea and vomiting, pain in the abdomen, diarrhoea and want of appetite appeared. In some cases there was profuse perspiration, tachycardia and nocturnal emissions.

### SUMMARY AND CONCLUSION

1 In this series of cases, the commonest cause of opium taking was the association with and example of other addicts. Disease came next as a cause.

2 The majority (85 per cent) of addicts started taking opium before 40 years of age.

3 59 per cent took more than ten grams a day and 85 per cent over 5 grams a day.

4 The younger addicts showed a greater tendency towards taking larger doses than elderly persons.

5 Those who acquired the habit later in life remained satisfied with comparatively small doses.

6 The habit is easily acquired but difficult to break off.

7 65 per cent of the addicts declare that the habit is harmful. They want to give it up but are afraid to do so because of the withdrawal symptoms.

8 Opium addiction is rare amongst the women.

9 The withdrawal symptoms are identical with those observed elsewhere.

We are very grateful to Dr K Venkatachalam for his help in the analysis of the data collected.

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TABLE  
Mean Temperature and Humidity, Mercara

Months	Average mean monthly maximum tempera- ture	Average mean monthly minimum tempera- ture	Mean monthly relative humidity at 8 hours			Mean monthly relative humidity average of preceding 3 years
			1924	1925	1926	
January	77.3	56.7	70	75	81	78
February	80.9	58.8	67	64	75	68.6
March	84.5	61.3	74	70	68	70
April	85.8	62.1	80	87	68	78.3
May	80.1	64.2	88	90	84	87.3
June	72.3	63.1	94	92	97	94.3
July	68.9	61.1	97	96	96	96
August	69.4	61.8	96	97	97	97
September	71.9	61.6	92	92	95	93
October	75.5	62.1	86	83	86	87
November	73.5	58.1	86	83	79	82.6
December	75.2	57.5	88	91	73	84

monthly mean relative humidity which is not less than 60 per cent, and a mean monthly minimum temperature which does not fall below 56.7°F

It therefore conforms in respect of temperature and humidity to the above postulates for transmission, as we have seen, the suspected vector, *P. argentipes*, is present in abundance, and the parasite has apparently been seen in human hosts.

The country is not unlike the Garo Hills and the North Cachar Hills in Assam, localities in which Kala-azar prevails, and it would be interesting to know what reasons other than a fortuitous absence of the parasite, account for the immunity of Coorg from Kala-azar, but on this investigation, this was not what we went out for to see.

#### RECOMMENDATIONS

It is obvious that the secrets of the malariology of a hyper-endemic area like Coorg cannot be revealed by a few weeks' work, and it follows that the chief recommendation which one has to offer at this stage is to urge the desirability that more such work should be undertaken.

In regard to Mercara, however, the observation that *A. listoni* is bred in profusion by the open stone-pitched drains constructed by the municipality, and that it is replaced in swamps and natural water courses by other less efficient malaria carriers, suggests one of the few specific recommendations which the investigation at this stage warrants.



Stone-pitched cross drains breeding *A. listoni* and *A. culicifacies*,



Breeding grounds of *A. listoni*

We have shown that the stream which runs through Brahman Valley is throughout its course breeding *A. histom* in numbers, and it runs through a populous quarter which is heavily infected with malaria. There can be no doubt that it is very desirable that this stream, from some convenient point in its valley below Beauvoir—perhaps from below Mr Temmaya's house, to its junction above the Causeway with 'Fish River,' should be canalised.

A small masonry 'invert' should be provided to take the dry weather flow, and the sides of the drain should be made pucca sufficiently high up to take the maximum storm water flow.

It must in construction be so designed as to be able to resist damage from storm water, for if unsuitably constructed the sides may break down and the 'invert' be dissected out by a sudden heavy flow.

Weep holes may be required in marshy places along its course, and suitable junctions with house sullage drains must be provided. The considerable expenditure involved in this work should not be undertaken except under the advice, in regard to details of construction, of an engineer with experience of such work.

Such expert advice is also required for the solution of the problem of how the municipality should deal with the existing open surface drains which have been put down to drain swamps in Fish River Valley and elsewhere.

Such drains, as we have seen, substitute a profusion of a dangerous carrier mosquito for a smaller number of less efficient carriers and there would perhaps be a case for filling them all up and letting the land return to swamp again, as the cost of substituting underground subsoil drains would probably be deterrent.

Advice might be sought as to the probable effect from the point of view of drainage, of filling them up with stone and rubble, and then turfing them over, as by doing so some degree of drainage might still be effected, and at the same time, *A. histom* would be effectually excluded.

Possibly with the draining of the swamps, only some of these cross drains actually hold water in the spring months, which is the critical time, and if a detailed survey of Mercara in these months were to be carried out, it would then be possible to say which and how many of these drains should be put underground. At any rate, one would urge that under no circumstances should any more swampy land be drained by open surface drains 'herringboned' to a central open surface drain.

In such valleys, subsoil contour draining should be employed, and when the expense of so doing is likely to be out of proportion to the value of the land to be so reclaimed, then, in so far as these observations have gone, it would from a health point of view appear to be advisable to leave the swamps alone. Perhaps one may remark here that such considerations suggest that a malarial drainage expert may eventually be found to be a necessary member of an Imperial Anti-malarial Bureau.

#### JUNGLE CLEARANCE

Although the cut stumps of the trees in the plantain groves in Brahman Valley do not as it has been suggested, provide anopheline breeding grounds, as



a matter of general hygiene a clearance of these is desirable, for they almost certainly afford shelter to the adult mosquitoes, the larvae of which are to be found in the stream

No special measures are required in regard to wells, none of which were found to contain anopheles larvae

#### ANTI-MALARIAL HYGIENE AND PERSONAL PROPHYLAXIS

The senior writer, as an ex-public health official, at the risk, perhaps, of incurring the reproach that like a certain Biblical dog, he has returned to his old habits again, would nevertheless venture some comments on the hygienic aspects of the malarial problems of Coorg which were prompted by some five weeks' wanderings there

We have seen that Coorg with a splenic index of 63.3 per cent is a hyper-endemic area, that is to say, one in which malaria is very prevalent

If one takes into account the nature of the country, its abundant rainfall, its warm and humid atmosphere, its hill streams, its valleys moist with seepage water, and its abundant vegetation, one is forced to the conclusion that it is not in our stars that the complete eradication of malaria from such a country can be accomplished and that control and mitigation rather than abolition should be the goal of anti-malarial endeavour in Coorg

With one's thoughts running in this direction, one is astonished to note the absence of any personal anti-malarial prophylaxis in Coorg. As we have noted, the people are progressive, and receptive of new ideas. The children, scarcely deterred by almost universal splenic enlargement and frequent attacks of fever, fill the well-appointed schools of the province, European clothes, foods, liquors, and wares, are on sale in all the bazars, and a network of motor transport covers the roads of the province

Yet in only one of the many Coorg houses entered in the course of this investigation was a mosquito net seen, and that in the house of a Forester, who had been taught its use at a Forest School. The reason given for their absence is that mosquito nets are too expensive for family use

In the senior writer's experience, in other parts of India, cheap nets made of a coarse muslin or such like fabric, are sold at a price of some three or four rupees, and are universally used

In Coorg, however, although almost every bazar contains a wine shop in which imported spirits are for sale, and the wine merchant is a man of substance, the mosquito net is considered an expensive luxury, and it is hardly ever used, although it should be possible to procure two nets for the price of a bottle of whisky

It is evident that the education of the Coorg in personal anti-malarial prophylaxis has been surprisingly neglected

It behoves the inhabitants of a country, which is, malarialogically, a hyper-endemic area, to live hyper-endemically, so to speak

Some well directed anti-malarial propaganda is badly required to teach the Coorg how to protect himself against the ubiquitous malaria-carrying anopheles of his country



# STUDIES ON LATHYRISM

## Part II.

BY

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ANDERSON, HOWARD AND SIMONSEN (1924\*), as a result of their studies on Lathyrism, of which the present paper is a continuation, came to the following conclusions —

- 1 'The seeds of *khesari* (*Lathyrus sativus* L.) have been found, on chemical examination, to be free from substances of an alkaloidal nature. Controlled experiments with this seed over long periods, with ducks and monkeys have demonstrated that the grains are harmless and provide a nourishing diet for these animals.
- 2 An examination of the weeds which contaminate *khesari*, has proved that *akta* (*Vicia sativa* L. var *angustifolia*) contains bases showing alkaloidal properties. Two such bases, vicine and divicine, and a cyanogenetic glucoside vicianin, have been isolated, prepared in the pure state, and used in inoculation experiments on animals. Divicine which occurs in *akta* in combination with a sugar, as the glucoside vicine, produces on inoculation in guinea-pigs a characteristic and fatal disease. *Akta*, when fed to ducks, causes death. In monkeys, it produces a very characteristic train of symptoms affecting the nervous and muscular systems.
- 3 Though certain of the symptoms occurring in monkeys fed on diets containing *akta* have been described in cases of human lathyrism we are not yet in a position to state, in the absence of pathological proof, that *akta* is the cause of lathyrism in man.'

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\*Ind Jour Med Res, Vol XII, 1924-1925, p 613-643

Such work might be attempted by a series of popular lectures in suitable centres, and by adding a series of school lessons on malaria and personal protection to the school curriculum

The manufacture, popularisation, and distribution of mosquito nets might perhaps be undertaken by the ladies of the provincial Red Cross Society

It is evident that in this line of work there would be much scope for the activities of a provincial health staff, but in regard to this the Director of Public Health, Madras, has recently advised the Coorg administration, and there is no need for further elaboration of the theme by us

### QUININE, FEBRIFUGE, AND CINCHONA

It will be seen from the figures supplied by the Civil Surgeon that the amount of quinine supplied by the Coorg Government for its dispensaries, for free distribution in its schools, and for sale by official agencies, is a generous provision, being 17 grains per head of the population (Report of the Coorg Malaria Committee 1926), and that there is no lack of appreciation of its utility, but the fact that they could themselves grow it does not appear to have been put to practical application

Every schoolboy knows that in nature stinging nettles and dock leaves, the poison and its antidote, grow side by side, every Coorg schoolboy should know that so also might it be in Coorg with regard to malaria and its remedy

From the official records cited as references, it appears that *Cinchona succirubra* does well in Coorg

As long ago as 1863 there was a plantation of *C succirubra* three miles east of Mercara, which had several hundred flourishing trees on it

We have not been able to trace its subsequent history or its exact location, but its fate was probably determined by the desuetude into which the cultivation of *C succirubra* has fallen owing to the fashion in medical practice which led to the rejection, in favour of quinine, of the other alkaloids of the bark

As *C succirubra* is comparatively poor in quinine, although rich in the other alkaloids, *Cinchona ledgeriana* and a hybrid between *C ledgeriana* and *C succirubra* have replaced the latter in commercial cinchona cultivation

It appears that *C ledgeriana* has been cultivated in Coorg as a side-line to coffee growing, as it was hoped that the tree would yield good shade for the coffee bushes, and that the bark would repay collection

In the Report on the Economic and Material Progress of Coorg 1826—1902, it is recorded that 'by the commencement of the decade, the cinchona industry was already dead, the trees yield poor shade, and at the prices then prevailing it did not pay to send the bark home'

The trees were therefore rooted out, and the cultivation of *Cinchona* was abandoned in the 'nineties'

Despite the improvement in prices since these days, the industry has not been revived, for economic causes with which we are not herein concerned

The point we wish to make is that the *Cinchona* tree, and particularly the hardy *C succirubra*, will grow and flourish in Coorg.

## OBJECT OF THE RESEARCH

The object of the investigation, with which the present paper deals, was to learn whether or not a morbid state resembling Lathyrism could be produced in rats by means of diets into which *khesari* or *akta* entered largely

For this purpose, the following samples of these grains were used —

- (1) A large-seeded, deep-rooting type of *Lathyrus sativus*, grown by cultivators in Broach This seed was obtained from Mr Albert Howard, Director of the Institute of Plant Industry, Indore It was carefully cleaned and reported by him to be a 'pure culture free from akta'
- (2) A large-seeded type of *Lathyrus sativus*, grown by cultivators in Rewah This seed was obtained from Lt-Col T C McCombie Young, I M S, who collected it 'with much difficulty from cases (of lathyrism) who had been eating it prior to getting lame' The sample contained approximately 10 to 15 per cent of *gram* (*Cicer arietinum*), much of it was weevilled
- (3) A pure culture of *akta* (*Vicia sativa*) grown by Mr Howard, from selected seed, at the Institute of Plant Industry, Indore

EXPERIMENTS WITH *Lathyrus sativus*

*First experiment*—In this experiment the pure culture of *Lathyrus sativus* was used Eleven groups of six young rats, of the same aggregate body-weight, were fed as follows —

Group I	on whole wheat flour, 100 per cent				
II	"	"	"	90	" plus lathyrus 10 per cent
III	"	"	"	80	" " 20 "
IV	"	"	"	70	" " 30 "
V	"	"	"	60	" " 40 "
VI	"	"	"	50	" " 50 "
VII	"	"	"	40	" " 60 "
VIII	"	"	"	30	" " 70 "
IX	"	"	"	20	" " 80 "
X	"	"	"	10	" " 90 "
XI	on pure lathyrus 100 per cent				

Each animal was confined in a separate cage under conditions of scrupulous cleanliness Water was provided for drinking and washing purposes, and sterilized cotton waste as bedding The proportion of males to females was the same in each group The animals were allowed to eat as much of their respective diets as they liked, they were weighed weekly The experiment commenced on the 7th January and ended on the 17th May, 1927, (130 days) During the period from 19th January, 1927 to 7th February, 1927 (20 days), each group was given buttermilk All animals dying during the course of the experiment were subjected to post-mortem examination

The results of the experiment are shown in Figs 1 to 11 None of the 66 animals fed on diets containing varying proportions of lathyrus developed any disease of the nervous system Five died of pneumonia (L 2, 5, 28, 39 and 62,

We have now changed our minds about the other alkaloids of Cinchona, and competent authorities hold that these alkaloids other than quinine, which are contained in Cinchona febrifuge, are probably as effective in the cure of malaria as is quinine.

Major J. A. Sinton, V.C., O.B.E., I.M.S., has been good enough to place his file on the subject at our disposal and he authorises us to quote him as being of the opinion that for ordinary routine cases of malaria in dispensary practice, Cinchona febrifuge when given by the mouth is as effective a cure for malaria as quinine is, and he advises that in his experience the febrifuge is not more likely to produce unpleasant symptoms of nausea than is quinine.

Here, then, is a tree which might be grown in every Coorg's back garden so to speak, from the bark of which by a comparatively simple process an effective cure for malaria can be prepared.

These possibilities have already appealed to a former civil surgeon of Coorg, Lieut-Col F. C. Fraser, I.M.S., who, in a letter to the Commissioner of Coorg dated 25th January, 1924, advocating the promotion by Government of 'limited scattered cultivation of Cinchona,' urged that 'an infusion made from the bark would appeal to the Coorg, as it is going back to primitive or Ayurvedic medicine, and such a decoction would be as effective as the febrifuge now employed.'

In considering these possibilities, it becomes evident that Government action would be required to initiate this policy. From the tables which show the amount spent annually in the purchase of quinine and febrifuge for use in hospitals and dispensaries, and for sale in the province, the question arises as to whether, as a business proposition, with large areas of Government forest land available, and with a forest department to handle the products, it would not pay the Coorg Government to make their own Cinchona febrifuge for provincial use.

Even if the work were not actually remunerative, a small loss might be written off as cinchona cultivation propaganda.

Once the Coorg learned from actual demonstration how easily the red bark could be grown from which a febrifuge or a decoction could be produced, one might hope to see in very fact the poison and its antidote growing side by side, the ravines and valleys producing the malaria-carrying anopheles, and on the slopes above them the cinchona tree.

Turning from such visions to practical considerations, the following facts, which have been culled from literature on the subject, may be useful in this connection.

From an editorial note in the *Indian Medical Gazette*, July 1912, one learns that one-year seedlings are planted out 1,000 to 2,000 of them per acre. The bark is harvested when the trees are ten years old. The tree may be uprooted for harvest so as to allow of collecting the root bark as well as that of the trunk, or 'coppicing' may be adopted, in which the root is left, from which new stems spring up (MacGilchrist, 1914-15) describes the preparation of the febrifuge as follows —

'The bark of the roots trunk, and stems is chipped off, dried and powdered. The powdered bark is mixed with lime and just sufficient water to make it damp, the lime

Figs 1, 5, 7 and 11), one died of enteritis (L 38, Fig 7); the remainder survived. The sample of pure *Lathyrus sativus* used in this experiment was incapable of causing lathyrism in rats within a period of 130 days. In nutritive value it was superior to whole wheat flour (*cf* Figs 1 and 11). Its vitamin-B-value, as determined by biological assay on rats, was comparatively high being only slightly less than whole wheat.

At the end of the experiment the five survivors in Group XI, which had been fed for 130 days on whole lathyrus, and the 16 survivors in a second experiment (*vide infra*), which had been fed for 117 days on whole lathyrus, were placed together in one large cage and their exclusive diet of pure lathyrus continued for a further period of 60 days. In no case did a condition resembling lathyrism, nor any disease of the nervous system, arise, the animals remaining in extraordinarily good health despite that they have eaten nothing but pure lathyrus for over 6 months. Cannibalism did not occur. The reproductive capacity of the animals was not appreciably impaired, a number of litters being born during this period of 60 days.

*Second experiment*—Eighteen young rats were used in this experiment. They were fed from the outset on cakes composed entirely of the pure *Lathyrus sativus*. As in the first experiment, each animal was confined in a separate cage under conditions of perfect hygiene. Water was supplied in abundance. The results of the experiment, which lasted for 117 days, are shown in Figs 12, 13 and 14. They confirm those of the first: in no case did lathyrism, nor any disease of the nervous system occur. Two of the 18 animals died during the course of the experiment, post-mortem examination did not reveal the cause of their death.

*Third experiment*—In this experiment the *khesari* eaten by human sufferers from lathyrism prior to the onset of the disease, was used. Eighteen young rats, each confined in a separate cage under conditions of perfect hygiene, were employed. They were fed from the outset on cakes composed entirely of this *khesari*. Water was provided in abundance. The results of this experiment, up to the 136th day, are shown in Figs 15, 16 and 17. They confirm those of the first and second experiments: in no case did lathyrism, nor any disease of the nervous system occur. Two of the 18 animals (L 85, 90, Fig 15) died during the course of the experiment: one from pneumonia, the other from an unknown cause. This experiment, commenced on 8th April, 1927, was in progress on 14th September, 1927, without any animal having developed lathyrism nor any disease of the nervous system\*.

#### EXPERIMENT WITH *Vicia sativa*

In this experiment the pure culture of *akta* (*Vicia sativa*) was used. Twenty-four young rats were employed, each being confined in a separate cage under conditions of perfect hygiene. They were fed from the outset on cakes composed entirely of *akta*, water being supplied in abundance. The results of

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\* This experiment was still in progress on the 21st December, 1927, without any of the animals having shown signs of lathyrism.—R. McC

being supposed to make the subsequent extraction of the alkaloids easier. The dampened mass is kept in buckets for a couple of days and then made into a thick paste with water and put into vats.

Caustic soda and mineral oil being added, the vats are heated up to 170°F, and the contents stirred for half an hour.

The contents of the vats are allowed to settle for an hour, when two layers form, oil on the top, and the exhausted bark sludge below. The oil, which contains all the alkaloids, is decanted off into separators, and mixed with dilute sulphuric acid. No artificial heat is applied to the separator, but the oil is still hot from the vats and the addition of sulphuric acid to water generates some heat. The mixture in the separators is allowed to stand for two or three hours by which time two layers form. The acid liquor, containing quinine, together with the other alkaloids, is drawn off into troughs. If this acid liquor has been prepared from *C. succirubra*, all the alkaloids it contains are precipitated with caustic soda and Cinchona febrifuge is obtained as amorphous alkaloids.

From this account it will be seen that no expensive plant is required for the production of febrifuge from the bark.

Subject to expert advice from those personally conversant with the business of febrifuge production, one would be inclined to think that the production in Coorg of febrifuge for provincial needs is a proposition which is within the range of practical politics, more especially as its main object would be that of demonstrating how a cheap remedy for malaria could be placed at the disposal of a population more than half of whom are, to judge from the spleen rates, permanently in need of it.

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the experiments, up to the 107th day, are shown in Figs 18, 19, 20 and 21. The rate of growth was poor, some animals growing fairly well, others badly. In no case did lathyrism, nor any disease of the nervous system occur. Two animals died during the course of the experiment: one from pneumonia, the other from an unknown cause. This experiment, commenced on 7th May, 1927, was still in progress on 14th September, 1927, without any of the animals having developed disease of the nervous system\*.

#### CONCLUSION -

Neither the pure culture of *Lathyrus sativus* (*Khesari*) nor of *Vicia sativa*, (*akta*) nor the *khesari* used by sufferers from Lathyrism in Rewah caused Lathyrism or any disease of the nervous system in rats.

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\* This experiment was still in progress on the 21st December, 1927, without any of the animals having shown signs of lathyrism.—R. McC.

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## CHART I

### DESCRIPTION OF FIGURES

- Fig 1 Weight curves of six young rats fed on an exclusive diet of whole wheat flour Two (L 2 and L 5) died of pneumonia, the remainder survived
- „ 2 Weight curves of six young rats fed on a diet consisting of 90 parts of whole wheat flour and 10 parts of pure *Lathyrus sativus* all survived
- „ 3 Weight curves of six young rats fed on a diet consisting of 80 parts of whole wheat flour and 20 parts of pure *Lathyrus sativus* all survived

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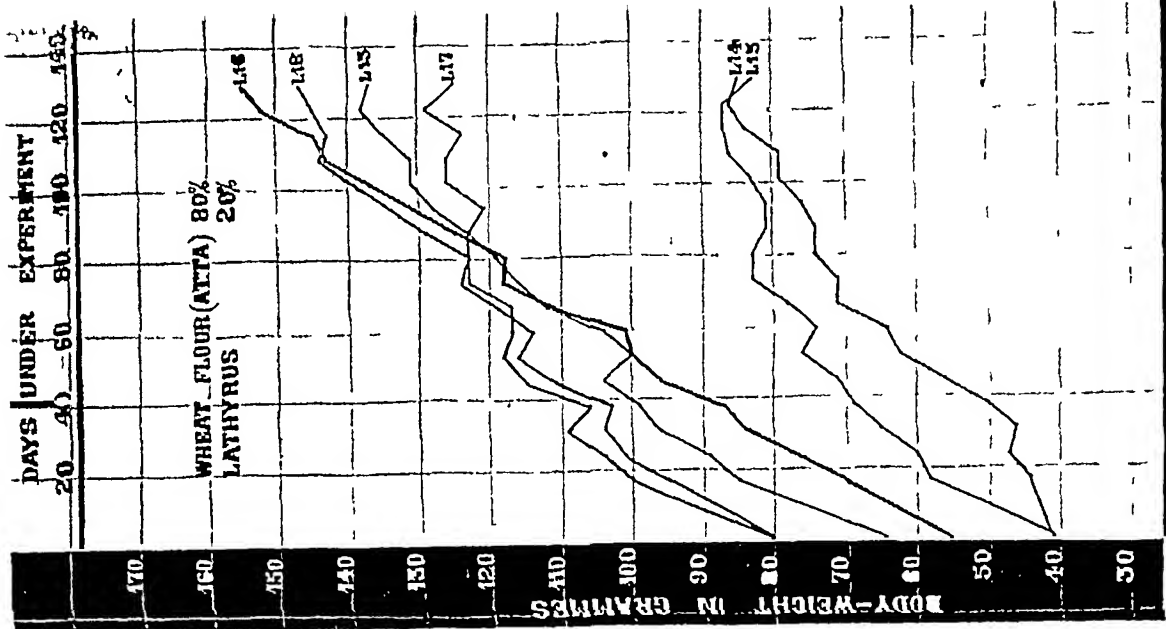


FIG 3

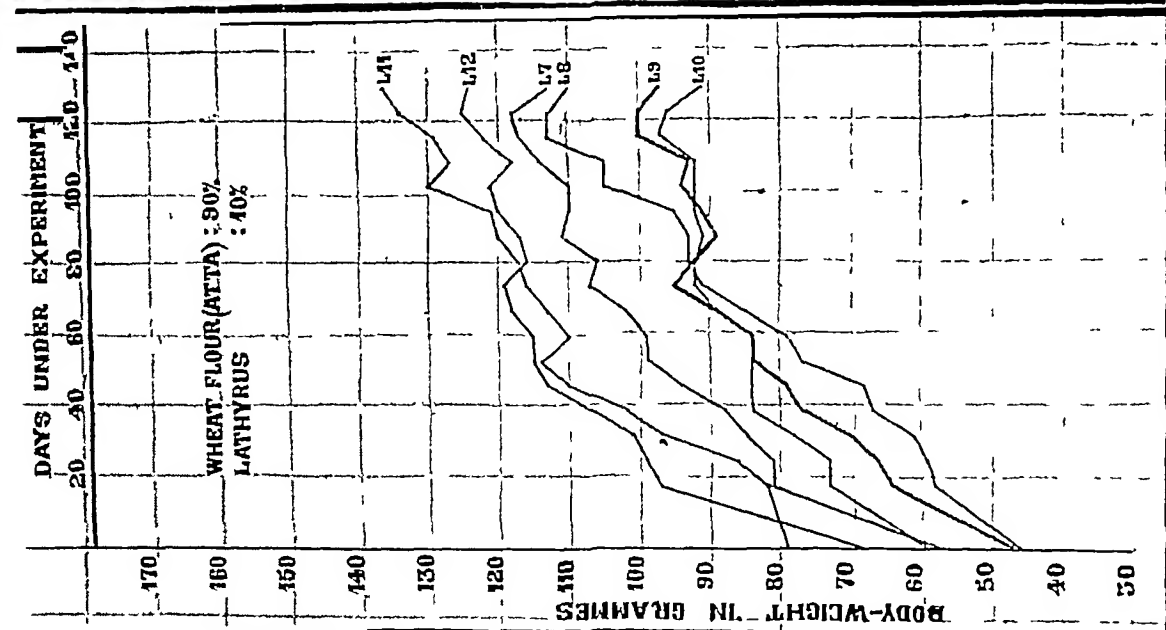


FIG 2

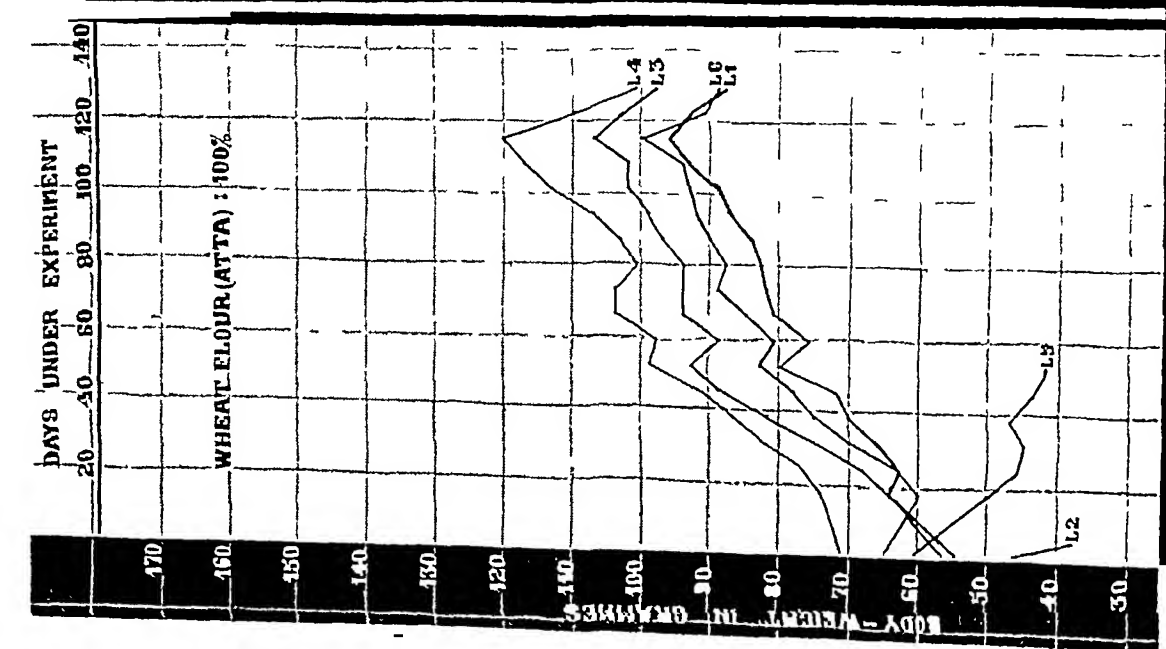


FIG 1



## CHART II

### DESCRIPTION OF FIGURES

- Fig 4 Weight curves of six young rats fed on a diet consisting of 70 parts of whole wheat flour and 30 parts of pure *Lathyrus sativus* all survived
- „ 5 Weight curves of six young rats fed on a diet consisting of 60 parts of whole wheat flour and 40 parts of pure *Lathyrus sativus* one (L 28) died of pneumonia; the others survived
- „ 6 Weight curves of six young rats fed on a diet consisting of 50 parts of whole wheat flour and 50 parts of pure *Lathyrus sativus* all survived

# EPIDEMIC DROPSY ITS BLOOD PICTURE, GENERAL AND BIOCHEMICAL

BY

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[Received for publication, February 14, 1927]

WITH the passing away of the monsoon in 1926, Calcutta and various other parts of Bengal were visited by an epidemic disease which, according to some, is beri-beri, while others termed it epidemic dropsy. It is rather difficult to decide on this difference of opinion. So far as beri-beri is concerned one can definitely say from clinical studies that it is certainly not the text-book beri-beri. The characteristic 'thung' of the Chinaman, the neurites, the foot drop, were all absent. The knee jerk was almost always present. The peculiar pallor of the pharynx and the loss of pain sensation when the calf muscles are pricked with a needle, which are characteristic of beri-beri (Col MacGilchrist), are not present in any of the cases studied. In beri-beri, the pathological change is mainly in the nervous tissue, whereas, in this condition, it is the cardio-vascular system that suffers most. So, without giving for the present any particular name to the disease, it will be desirable to record its principal features.

## SYMPTOMATOLOGY

*Gastro-intestinal*—The first symptom noted in many of the cases was some gastro-intestinal trouble. It was generally diarrhoea—flatulent or explosive—or dysentery, though a marked constipation was noted in a few cases. It has been noted that gastro-intestinal symptoms often varied inversely with cardiac symptoms. In severe cases, diarrhoea brought relief while constipation was accompanied by cardiac symptoms.

*Œdema and Erythema*—Though, usually, the œdema appears after a few days of gastro-intestinal trouble, yet there were a good number of cases where the œdema was the very first sign noted. It was more marked in the lower extremities than in the upper and pitted on pressure. In some cases it affected the whole body, even the scalp not excepted. Erythema of the œdematous skin was a common feature. General redness—a mottled redness—sometimes purple,



dark mottled blue were all noted in different cases. Sometimes scattered areas of dilatation of capillary vessels would lead to painful and bleeding ulcers or pemphigoid eruptions. In some cases there were angiomatous growths all over the body. Very painful piles—internal and external—were noted in many of the cases.

*Heart*—Most of the fatalities were due to heart-failure. Palpitation, throbbing, anginal pain were the usual complaints.

*Lungs*—The lungs were involved—generally bronchitis, and less often, broncho-pneumonia simulating influenza, were noted. Pulmonary œdema during failure of compensation and acute œdema with incessant cough and expectoration of large quantities of sanious sputum suggesting acute failure of the right heart, were the most distressing symptoms in many of the fatal cases.

*Kidneys*—Healthy.

*Liver*—The liver was generally found enlarged in cases of failure of compensation.

*Eye*—Glaucoma has been noted in some of the cases.

Since 1877-78, when it was recorded for the first time by Norman Chevers, the epidemic has visited this part of the country 3 or 4 times. The symptoms, as can be judged from the literature, were practically the same with some minor differences. One can rightly name epidemic dropsy as '*morbus Bengalensis*'—a name given by Chevers to a general rundown anæmic and æsthenic condition of the Bengalees due to insufficient dietary, since during every epidemic of this disease it is only the Bengalees—be they Hindus, Mahomedans or Christians—that suffered. Europeans, Marwaris and other communities, even when living in the very badly affected epidemic areas, have escaped altogether. In the case of Bengalees, it is only in the extremes of age that the disease is rare, certainly there is no age which is really immune. Cases have been noted even in suckling babies of dropsical mothers.

#### GENERAL BLOOD PICTURE

For the general blood examination samples have been collected at the same hour of the day from the patients on a fasting stomach—these precautions were taken with the idea of getting comparative figures and to avoid the physiological leucocytosis and other changes associated with digestion and absorption.

Table I gives the result of enumeration of the erythrocytes and leucocytes and also the findings of the percentage of hæmoglobin and the colour index.

From the table it will be evident that there is a marked diminution of the number of erythrocytes from that of the normal. The normal count in the Bengalees is 5,000,000 per c mm, whereas in this condition it may be as low as 1,500,000 per c mm.

With the diminution of the number of erythrocytes, the percentage of hæmoglobin has also come down and the colour index shows that there is a marked anæmia of the secondary type. In calculating the colour index the normal percentage of hæmoglobin has been taken as 100 per cent, but it should be noted that in Bengalees in normal healthy condition, it is generally 80 per cent.

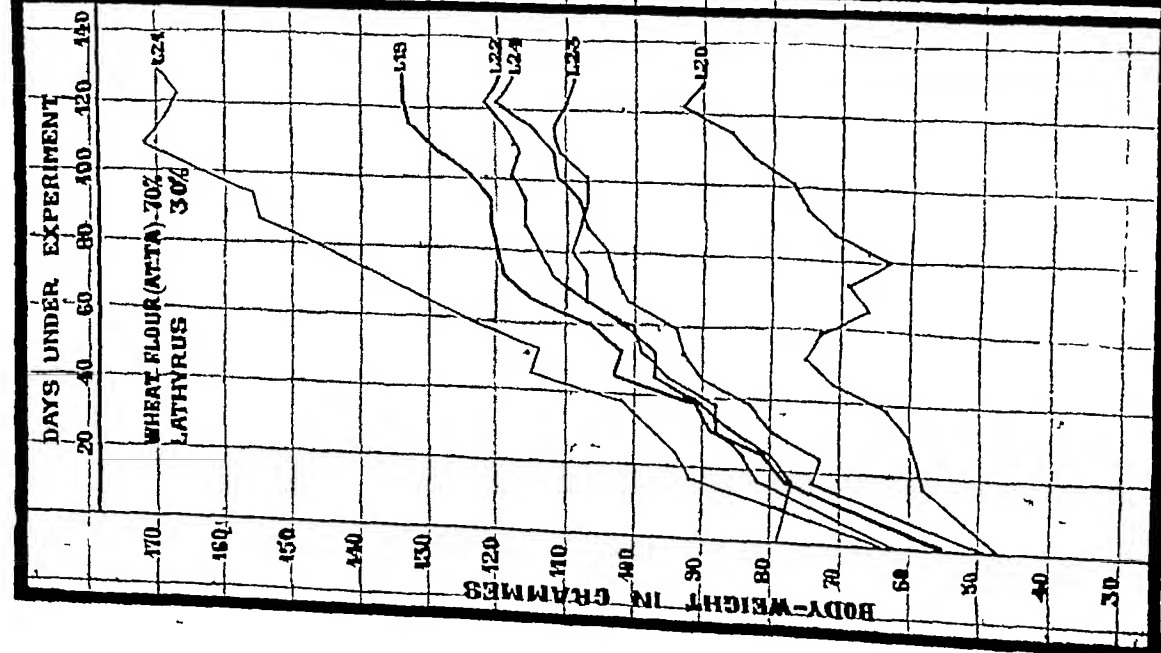


FIG 4

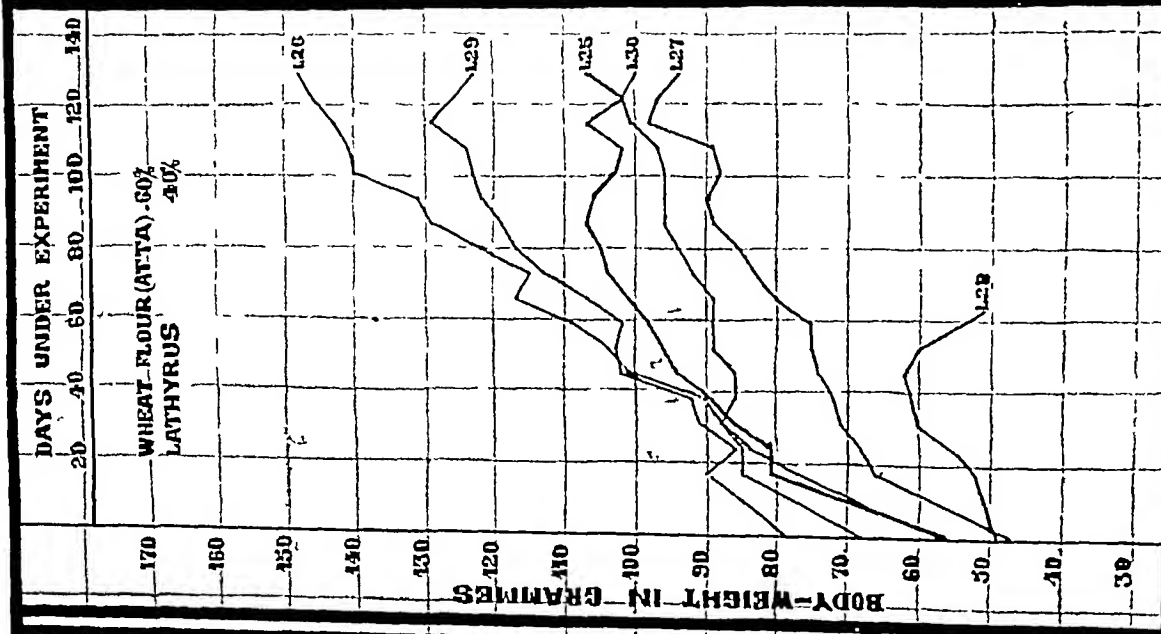


FIG 5

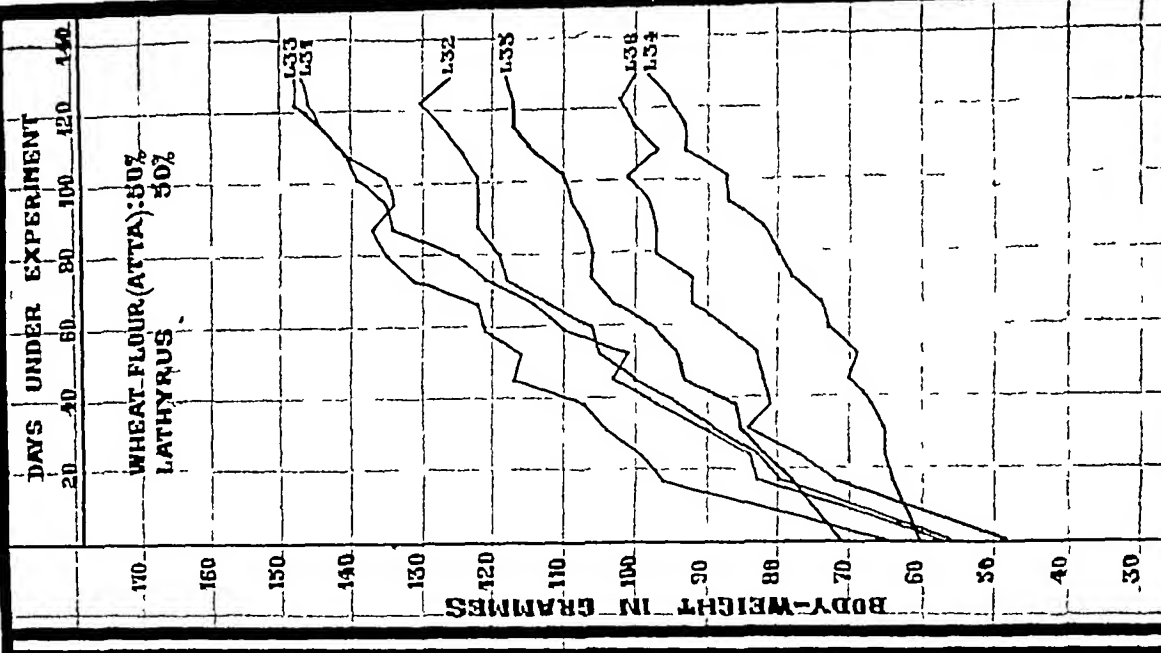


FIG 6

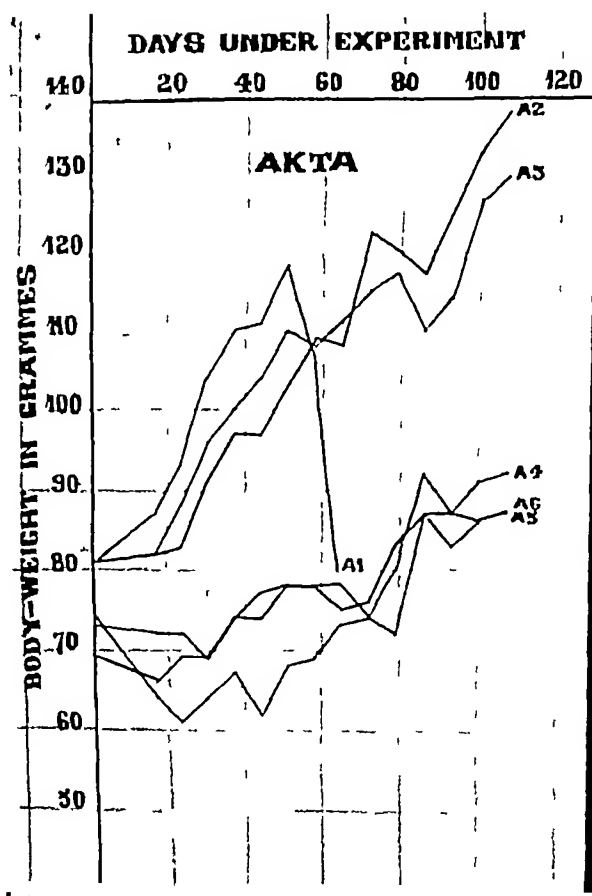


FIG. 18

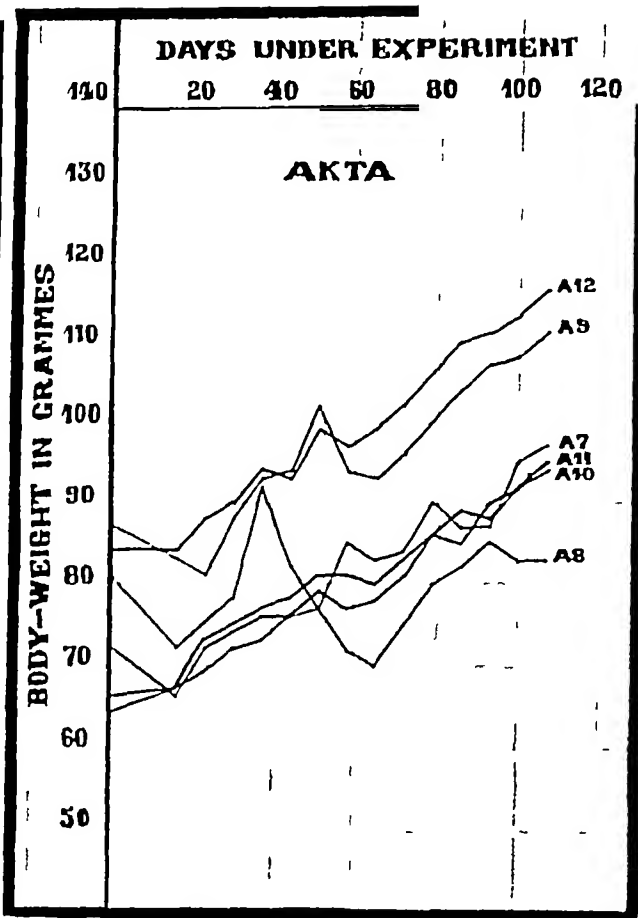


FIG. 19

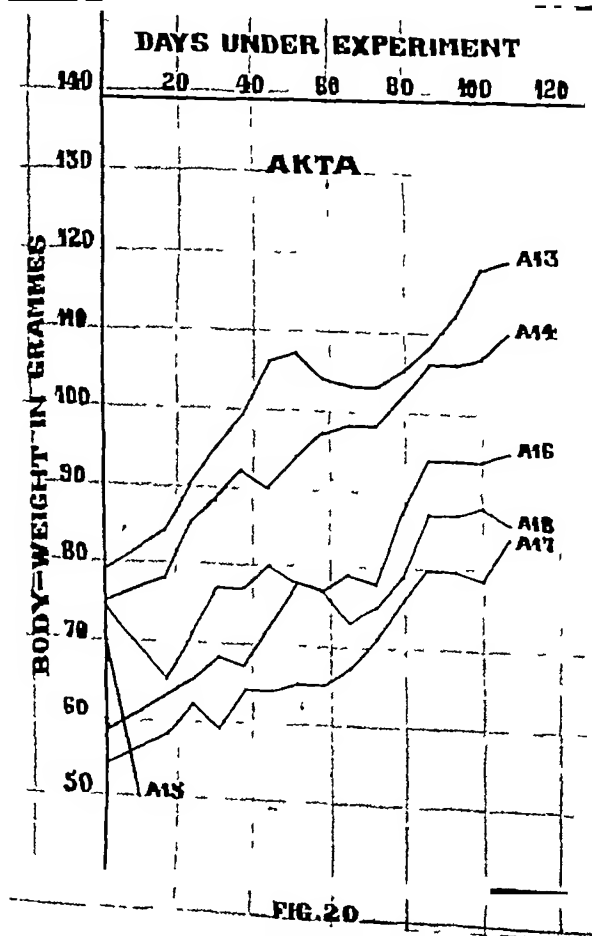


FIG. 20

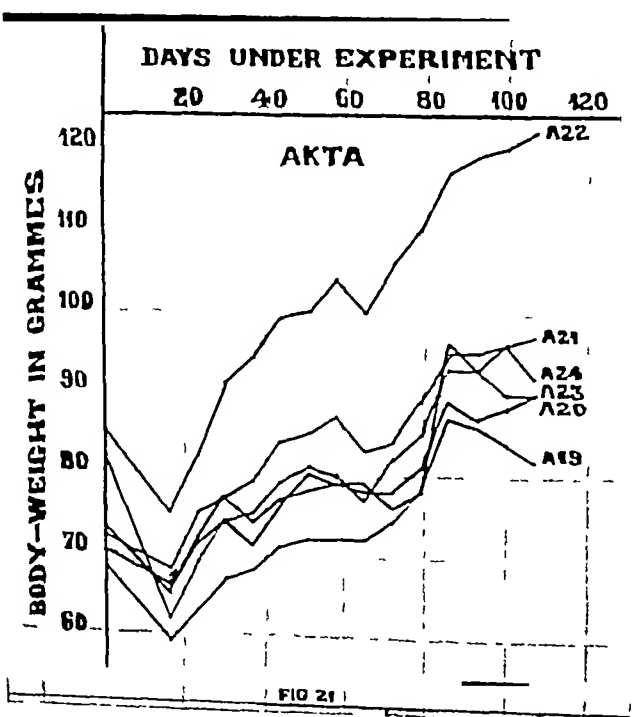


FIG. 21

### CHART III

#### DESCRIPTION OF FIGURES

- Fig 7 Weight curves of six young rats fed on a diet consisting of 40 parts of whole wheat flour and 60 parts of pure *Lathyrus sativus* one (L 38) died of enteritis, another (L 39) died of pneumonia, the remainder survived
- „ 8 Weight curves of six young rats fed on a diet consisting of 30 parts of whole wheat flour and 70 parts of pure *Lathyrus sativus* all survived
- „ 9 Weight curves of six young rats fed on a diet consisting of 20 parts of whole wheat flour and 80 parts of pure *Lathyrus sativus* all survived



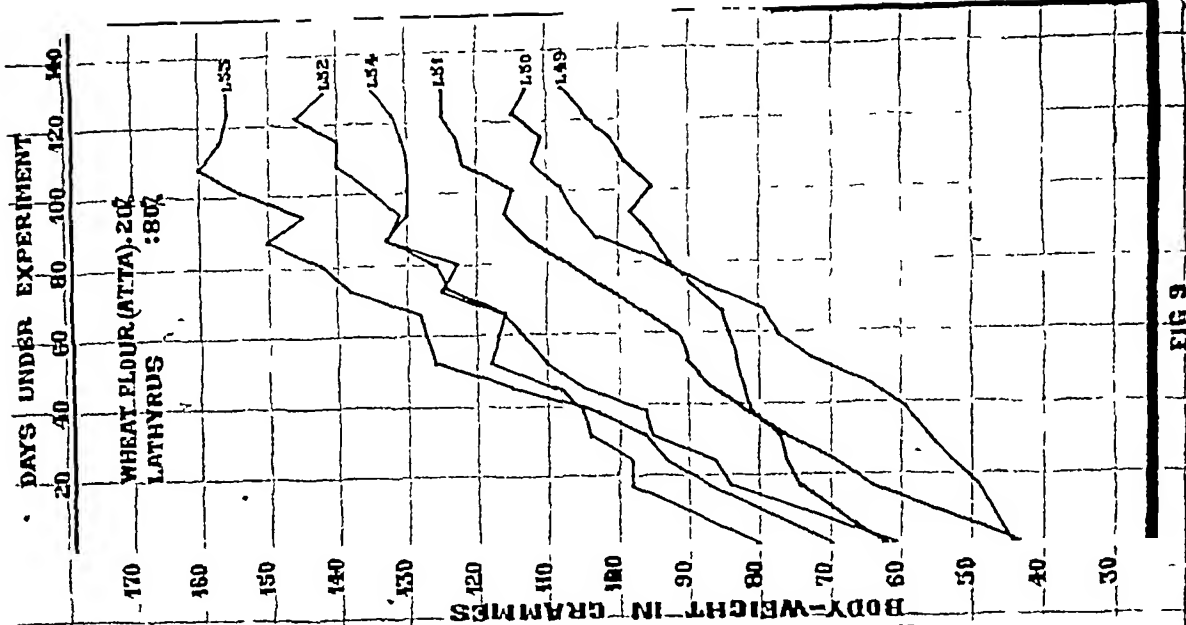


FIG 9

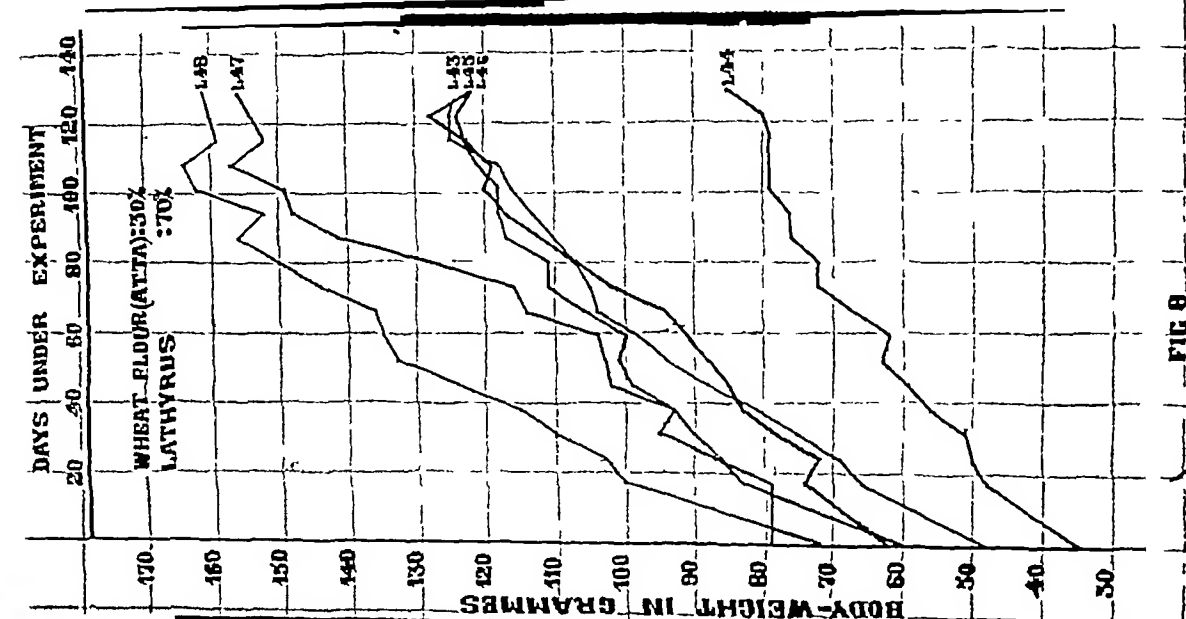


FIG 8

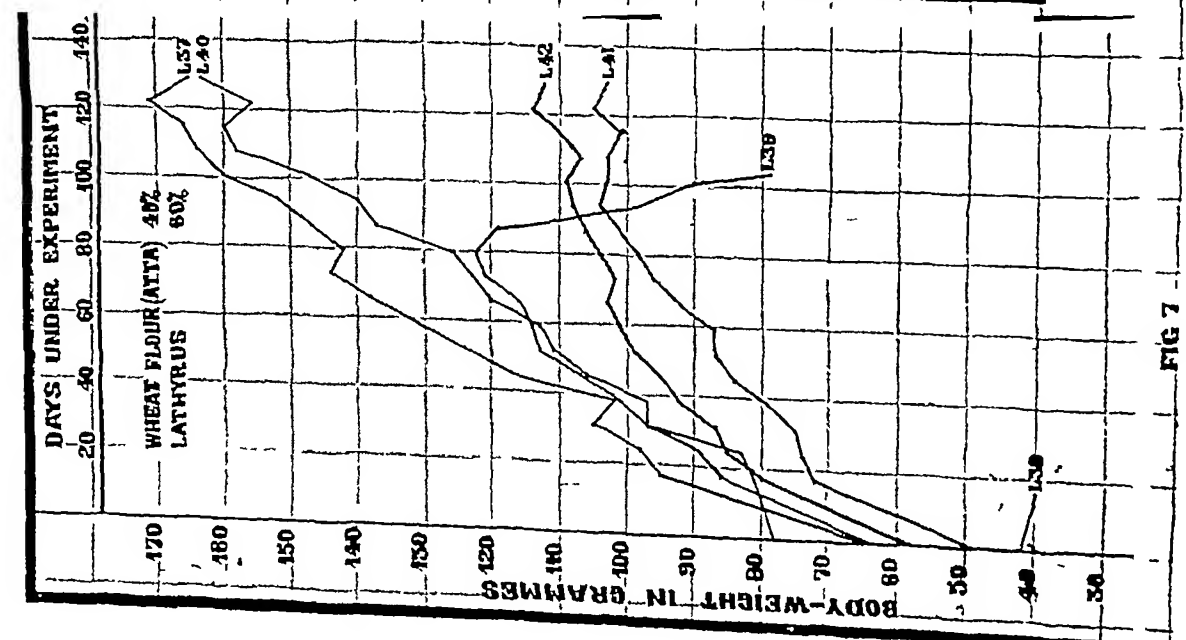


FIG 7

# THE EXPERIMENTAL PRODUCTION OF STONE-IN-THE-BLADDER

## Part IV.

BY

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[Received for publication September 26 1927]

IN previous Reports on this subject (1, 2, 3) the production in rats of urolithiasis and its sequelæ, by means of diets consisting of whole wheat flour (*atta*) or oatmeal, linseed meal, cornflour, calcium phosphate, common salt and water, and its prevention by the addition of milk to these diets, have been recorded. It has lately been found that stone-in-the-bladder may also occur in rats surviving for long enough periods (120 to 130 days) on an exclusive diet of white flour. From this it would appear that urolithiasis is not due to any toxic quality possessed either by whole wheat flour, oatmeal or linseed meal, but that it is due to metabolic disturbances resulting from certain deficiencies in the experimental diets. The stone-producing effect of an exclusive diet of white flour had not been observed when experiments were begun to determine whether this effect might be due to some toxic quality of linseed meal or of linseed oil. It is the purpose of the present paper to record the results of these experiments.

### THE EXPERIMENTAL DIETS

These were three in number —

*The first* consisted of 78 parts of whole wheat flour (*atta*), 20 parts of linseed meal, 1 part of calcium phosphate, 1 part of sodium chloride, and water. It will be referred to as 'DIET A'.

#### CHART IV

##### DESCRIPTION OF FIGURES

- Fig 10 Weight curves of six young rats fed on a diet consisting of 10 parts of whole wheat flour and 90 parts of pure *Lathyrus sativus* all survived
- „ 11 Weight curves of six young rats fed on an exclusive diet of pure *Lathyrus sativus* one (L 62) died of pneumonia; the others survived
- „ 12 Weight curves of 18 young rats fed on an exclusive diet of pure *Lathyrus sativus* two (L 75 and L 78) died from unknown causes, the others survived



The second consisted of 90 parts of whole wheat flour (*atta*), 8 parts of linseed oil, 1 part of calcium phosphate, 1 part of sodium chloride, and water. It will be referred to as 'DIET B'.

The third consisted of 90 parts of whole wheat flour (*atta*), 8 parts of sesame (*gmgelly*) oil, 1 part of calcium phosphate, 1 part of sodium chloride, and water. It will be referred to as 'DIET C'.

Twenty-one young rats, aged between 60 to 70 days, were fed on each of these diets. The details of the experiments, as regards hygiene and caging of the animals, were as recorded in previous reports. The animals weighed between 60 and 86 grammes at the commencement of the experiment on the 29th December, 1926. Ten males and eleven females were fed on Diets A and B, eight males and thirteen females on Diet C. The experiment was continued for 257 days, ending on 14th September, 1927.

## RESULTS OF THE EXPERIMENT

The results of the experiments are given in the accompanying Tables (I, II and III). They may be summarized as follows —

- (1) Three animals fed on Diet A, developed urolithiasis a case incidence of 14.3 per cent. In all three gravel, but no formed stones, was present in the bladder. The condition was associated with chronic cystitis in two cases, in the third case no macroscopical evidence of cystitis was present. In one of the three the ureters were markedly dilated and necrotic areas were present in the kidneys, the other two showed no macroscopical evidence of pathological change either in the ureters or kidneys.
- (2) Seven animals fed on Diet B, developed urolithiasis, a case incidence of 33.3 per cent. Formed stones were present in the bladder in four of them. The stones ranged in weight from 12 to 253.6 mgrms, they occurred either as single or as multiple stones. In three cases gravel, but no formed stones, was present in the bladder, in one of these (No. 16, Table II) gravel was also present in the kidney. The condition was associated with chronic cystitis in four cases, no macroscopical evidence of cystitis was present in the remaining three. Dilatation of the ureters occurred in two cases, pyelitis or pyonephrosis in three.
- (3) Five animals, fed on Diet C, developed urolithiasis a case incidence of 23.8 per cent. Formed stones (weighing 17.6 and 20 mgrms respectively) were present in two of these, gravel in the remaining three. The condition was associated with cystitis in only one of the five cases, dilatation of the ureters was present in three cases and well-marked pyonephrosis in one.

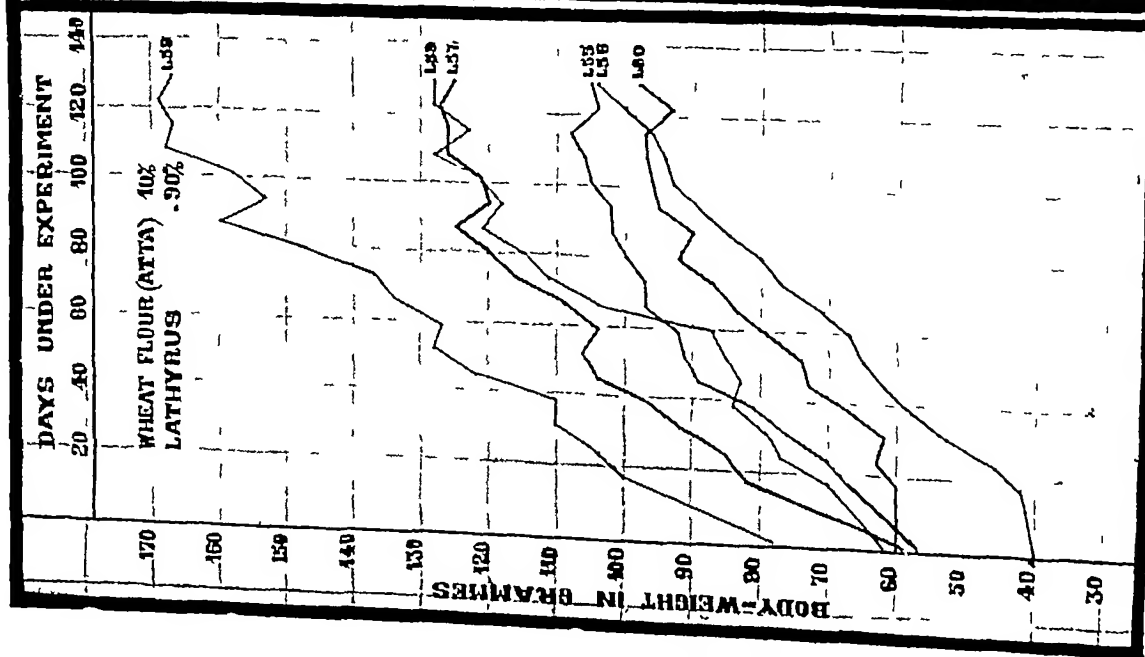


FIG.10

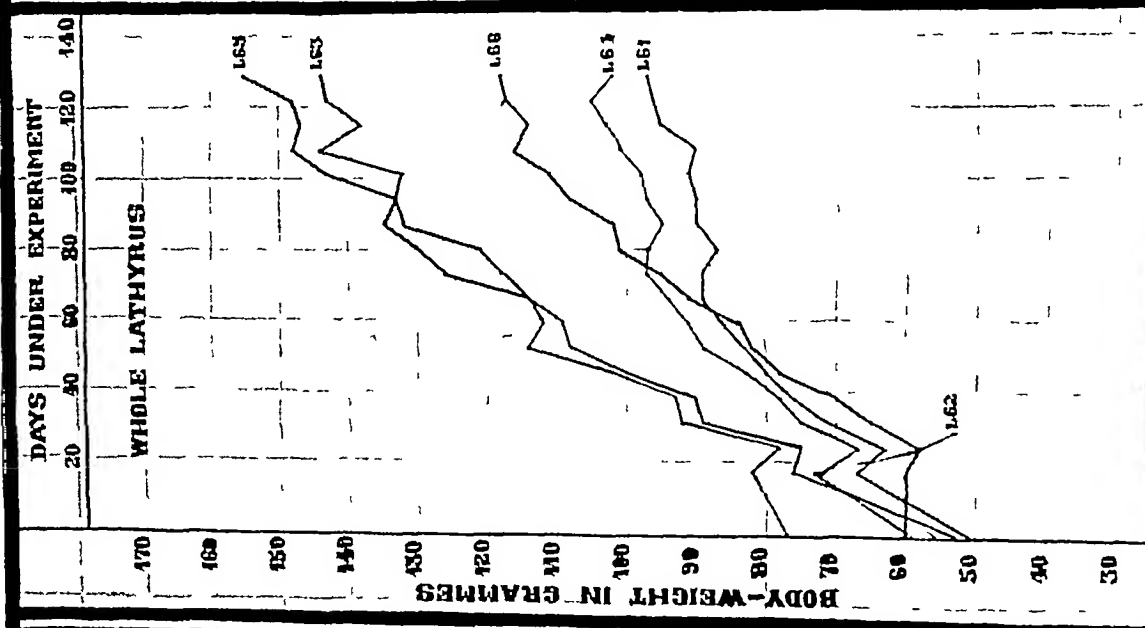


FIG.11

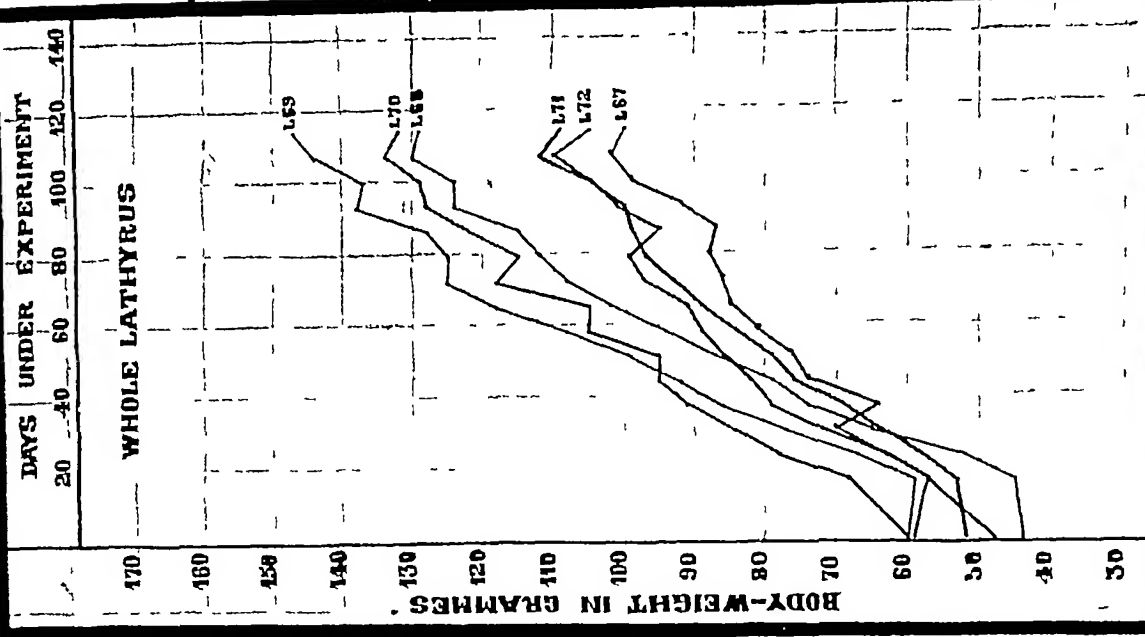


FIG.12

TABLE I

*Showing the incidence of urolithiasis and of its sequelæ, and the cause of death in 21 young rats fed on Diet A*

No	Sex	Original body weight	Final body weight	Days under experiment	Post mortem findings
1	M	68	134	117	Pneumonia
2	F	69	135	209	Pneumonia
3	M	78	154	161	Pneumonia
4	M	68	105	218	Anæmia
5	F	66	77	73	Pneumonia anæmia
6	F	68	93	126	Inflammatory growth in bladder
7	F	70	90	211	Anæmia
8	M	72	115	142	Pneumonia
9	M	71	112	168	Anæmia
10	M	74	105	194	Pneumonia.
11	M	69	80	94	Pneumonia
12	M	70	105	201	Pneumonia
13	F	65	110	97	Scurvy œdema
14	F	67	78	98	Anæmia
15	F	66	85	106	Gravel in bladder cystitis dilated ureters necrosis kidney
16	F	74	76	125	Pneumonia
17	M	79	140	183	
18	M	77	100	185	Anæmia
19	F	73	95	173	Gravel in bladder, no cystitis
20	F	71	115	179	Gravel in bladder cystitis
21	F	72	115	196	Pneumonia

## CHART V

### DESCRIPTION OF FIGURES

- Figs 13 and 14 Weight curves of 18 young rats fed on an exclusive diet of pure *Lathyrus sativus* two (L 75 and L 78) died from unknown causes, the others survived
- „ 15 Weight curves of 18 young rats fed on an exclusive diet of *khesari* used by sufferers from human Lathyrism one (L 90) died from pneumonia another (L 85) died from an unknown cause, the remainder survived

TABLE II

*Showing the incidence of urolithiasis and of its sequelæ, and the cause of death in 21 young rats fed on Diet B*

No	Sex	Original body weight	Final body weight	Days under experiment	Post mortem findings
1	M	72	115	163	Pneumonia
2	M	73	110	173	Anæmia
3	Γ	75	48	27	Enteritis
4	F	76	90	183	Pneumonia
5	M	70	93	151	Pneumonia
6	M	69	83	130	Pneumonia
7	F	66	75	231	Stone in-the bladder (95 mgrms) nephritis chronic cystitis
8	M	78	120	210	Stone in-the bladder (253.6 mgrms) chronic cystitis dilated ureters pyonephrosis
9	F	69	42	21	Enteritis
10	F	68	87	182	
11	F	67	59	102	Pneumonia
12	M	73	57	93	Pneumonia
13	M	72	80	101	Enteritis
14	M	74	90	198	Pneumonia
15	F	65	75	204	Gravel in bladder no cystitis
16	F	66	80	205	Gravel in bladder cystitis dilated ureters pyonephrosis gravel in kidney
17	F	67	70	212	Enteritis
18	M	83	48	15	
19	M	84	42	33	Stone in-the bladder (12 mgrms) no cystitis pyelitis
20	Γ	80	75	122	Stone in the bladder (21 mgrms) chronic cystitis
21	F	60	56	92	Gravel in bladder no cystitis

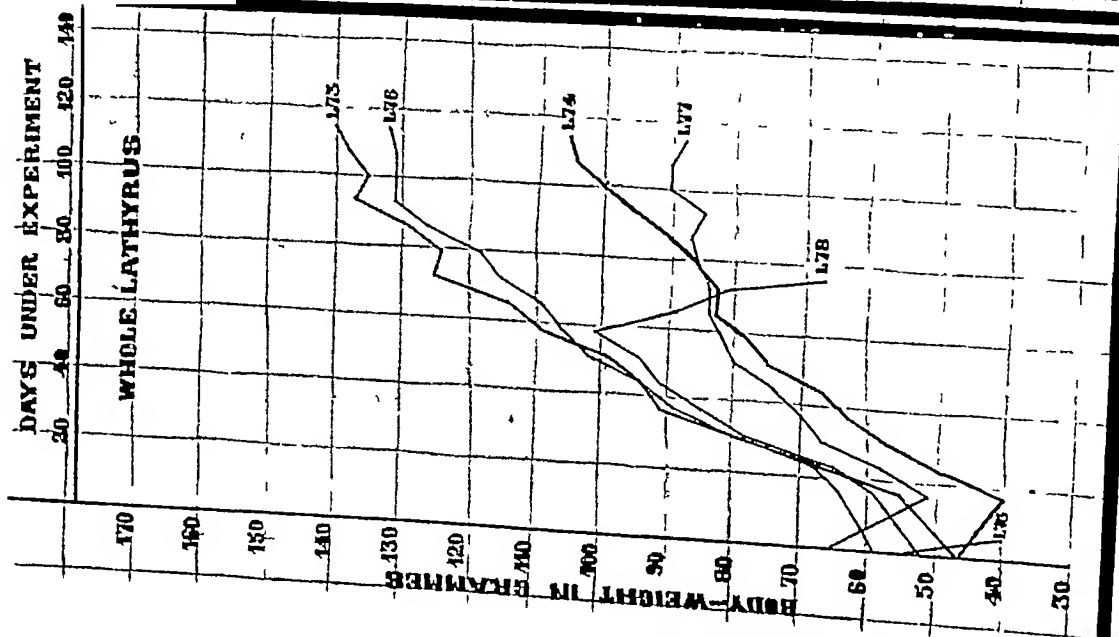


FIG.13

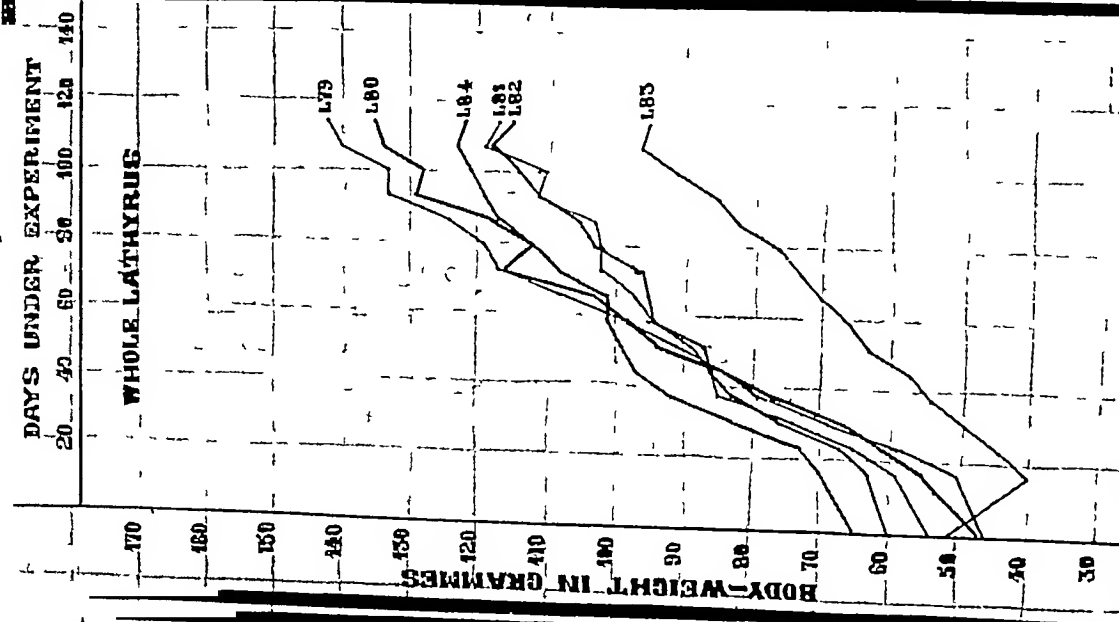


FIG.14

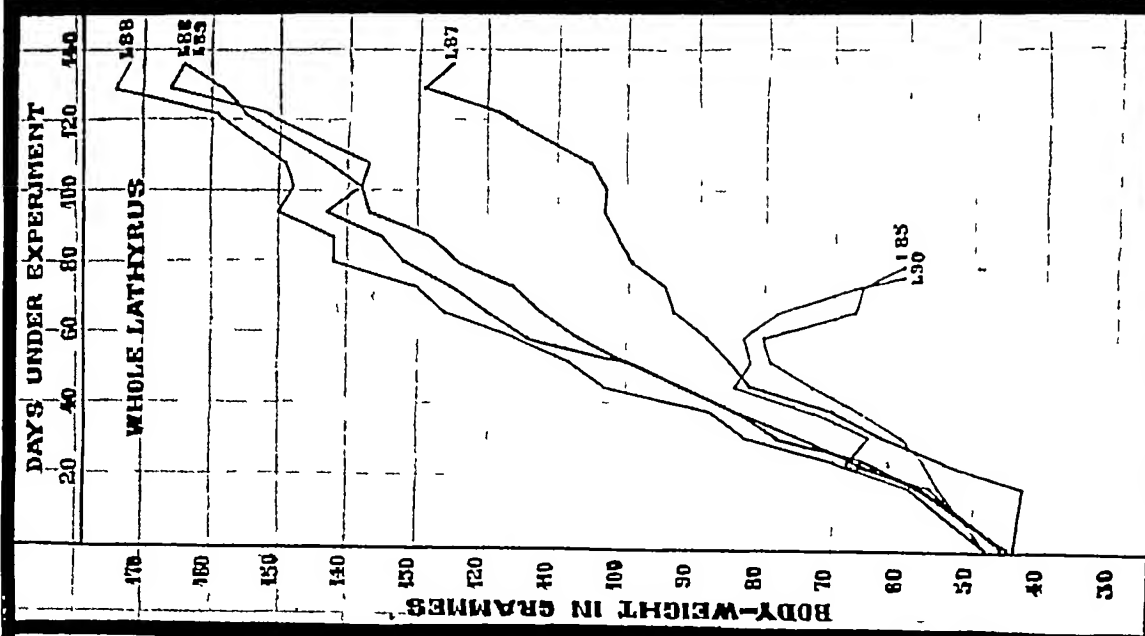


FIG.15

TABLE III

Showing the incidence of urolithiasis and of its sequelæ, and the cause of death in 21 young rats fed on Diet C

No	Sex	Original body weight	Final body weight	Days under experiment	Post mortem findings
1	M	69	113	181	Gravel in bladder dilated ureters no cystitis
2	F	70	145	187	Pneumonia
3	F	63	100	191	Gravel in bladder dilated ureters no cystitis
4	F	64	72	193	Stone in bladder (17.6 mgrms) no cystitis
5	F	65	95	246	Pneumonia
6	F	65	69	71	
7	F	66	84	195	Pernicious anæmia
8	M	62	117	175	Pneumonia
9	F	64	67	179	
10	M	76	96	154	Pneumonia
11	F	77	105	197	Anæmia
12	M	78	190	194	
13	F	65	100	93	Pneumonia
14	F	66	85	172	Anæmia
15	F	67	60	189	
16	M	84	90	184	Pneumonia
17	M	85	90	218	Gravel in bladder no cystitis
18	M	86	115	257	
19	M	80	93	175	Pneumonia
20	F	81	82	182	Anæmia
21	F	68	90	224	Stone in bladder (20 mgrms) cystitis dilated ureters pyonephrosis

Judging by the incidence of urolithiasis, the frequency with which formed stones were present, the size of the stones and the incidence of the sequelæ of stone, the diet composed of whole wheat flour and linseed oil (Diet B) was the worst of the three. But too much significance can not be attached to the difference in incidence of the disease in the animals fed on these three diets, for in different experiments, in which the same stone-producing diet (or what appeared to be the same) was used, the incidence of the disease varied within fairly wide limits. Thus, in a repeat experiment, carried out at the same time as the present one, in which 18 young rats were fed on a stone-producing diet consisting of

## CHART VI

### DESCRIPTION OF FIGURES

Figs 16 and 17 Weight curves of 18 young rats fed on an exclusive diet of *khesari* used by sufferers from human Lathyrism. one (L 90) died from pneumonia another (L 85) died from an unknown cause, the remainder survived



53 parts of whole wheat flour (*atta*), 20 parts of linseed meal, 25 parts of corn-flour, 1 part of calcium phosphate, 1 part of sodium chloride and water, the incidence of urolithiasis was 11.1 per cent although in a previous experiment (2) it was 22 per cent. There is, therefore, no significant difference between the stone-producing effect of Diets B and C. This effect does not seem to be due to any toxic action possessed by the oil of linseed as compared with that of sesame. But, as shown by the results of a previous experiment (3), it is due to the insufficiency in these vegetable oils of some substances (probably vitamin-A and probably not vitamin-D) provided in abundance by whole milk of good quality.

Of the 15 cases of urolithiasis in this series only seven were associated with macroscopical evidences of cystitis. Cystitis does not, therefore, appear to be a necessary forerunner of stone-in-the-bladder in rats, but, like pyelitis and pyonephrosis, it appears to be a sequel of stone. When the results of this experiment are taken in conjunction with those previously recorded (1, 2, 3), the following conclusions appear to be justified.

#### CONCLUSIONS

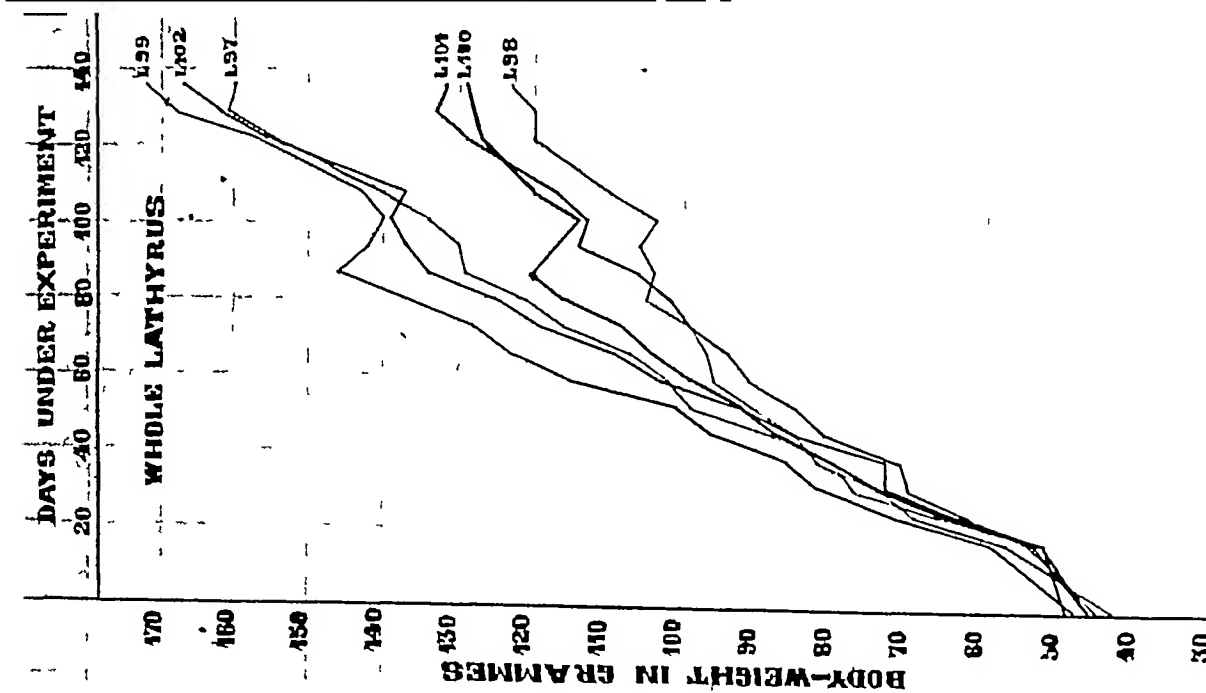
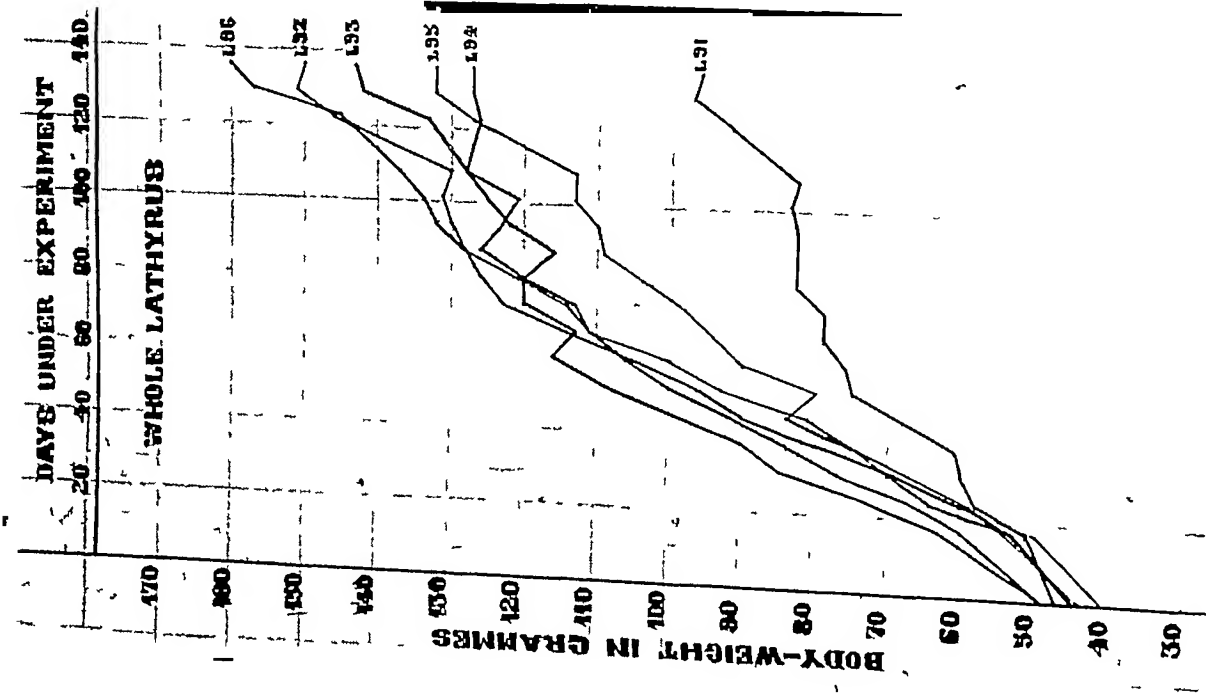
- (1) Stone-in-the-bladder in rats is due to the insufficiency in the experimental diets of a substance or substances (probably vitamin-A) which whole milk of good quality provides in abundance. This substance is not present in sufficient quantity in certain of the vegetable oils (linseed and sesame) in common use in India.
- (2) Cystitis is not a necessary forerunner of stone-in-the-bladder in rats.

#### REFERENCE

1, 2, 3 McCARRISON (1927)

*Ind Jour Med Research*, XIV, p 895, XV, p 197,  
and XV, p 485

CHART VI



## RAT LEPROSY

A RECORD OF EXPERIMENTAL WORK CARRIED ON AT THE  
SCHOOL OF TROPICAL MEDICINE AND HYGIENE,  
CALCUTTA, BETWEEN OCTOBER 1925  
AND AUGUST 1927

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[Received for publication, September 26, 1927]

THE bacillus of rat leprosy was first isolated by Stefansky(1) working in Odessa in 1903, and since that date it has been described by numerous workers as occurring in rats in England, the United States of America, France, Germany, Japan, Australia, New Caledonia and Brazil. The proportion of naturally infected rats varies considerably according to the findings of different observers. Marchoux and Sorel(2) working in Paris, systematically examined 1,296 rats and found evidence of infection in 65 (5 per cent), they state, however, that the percentage of positive findings varies in different localities and that as many as fourteen, nineteen or even forty-five per cent of the rats in certain lots may be infected.

The exact relationship between the human and the rat forms of leprosy is not certainly known, but there are certain factors common to the respective organisms which suggest a very close kinship: (a) Similarity in morphology and staining reactions; (b) Resistance to artificial cultivation; (c) The commonly intracellular position of the bacilli in both diseases; (d) The relative difficulty of transmission to experimental animals. With the exception of slight and transitory lesions in monkeys, laboratory animals have proved resistant to infection with the human strain and nothing approaching the generalised lepromatous condition found in man has ever been obtained. With the rat form, the

## CHART VII

DESCRIPTION OF FIGURES  
Figs 18, 19, 20 and 21 Weight curves of 24 young rats fed on an exclusive diet of pure *Vicia sativa* (*akta*) Their rate of growth was poor One (A 1) died of pneumonia, another (A 15) from an unknown cause, the remaining 22 survived

bacillus appears to be specific for this animal (to which it can, however, be transmitted fairly easily), experiments with monkeys, guinea-pigs, rabbits and Chinese hamsters have hitherto proved negative (e) The occurrence of rat leprosy bacilli in the superficial glands of apparently healthy rats (Marchoux quoted by Thibault) (3) Similar findings in the glands of otherwise healthy persons living in association with lepers have been described by Sorel (4), Conoy (5), and others (f) At least one case of rat leprosy occurring in a human being is reported in the literature (Marchoux) (6)

With this analogy before us, we deemed that a study of the disease in rats might prove of value in shedding light on the human lesions

Rats infected with leprosy were obtained in October 1925, through the kindness of Lieut-Col J Morison, I M S, Acting Director of the Haffkine Institute, Bombay, and with these as a basis of supply numerous experiments were set in train We propose to discuss briefly the more important of these Our post-mortem records are not so complete as we could have wished for two reasons (a) Animals dying during week-ends are frequently decomposed or partially eaten (b) Owing to an unfortunate mistake, commercial calcium carbonate contaminated with barium carbonate was administered to 12 rats from different deficiency-diet experiments shortly after these had been started all the animals rapidly succumbed as a result of this disaster A further difficulty with which one has to contend is the normal death-rate in any animal population rat leprosy is a disease of long incubation period relative to the normal expectation of life of this animal A proportion of rats die before there is time for the disease to develop

### I *Subcutaneous inoculation of rats on a full diet*

In all 52 rats on a full diet were inoculated subcutaneously in the groin Of these, 44 were subjected to post-mortem examination and lesions were found in 43 The negative rat was one which had been inoculated with a small dose from the original Bombay strain, the remaining 8 were not examined for one or other of the reasons mentioned above The *known* percentage of positive results is therefore 97.7 The shortest period after inoculation in which lesions were observed was 3 weeks and there was in a very general way a relationship between the interval which had elapsed since inoculation and the extent of leprosy involvement of the tissues, but this relationship was not a definite one At least two reasons can be advanced to explain this (a) differences in the resistance of individual rats to infection, and (b) variations in individual dosage The emulsions used in these experiments were standardised against standard opacity tubes, but as the source of the emulsion (in the present state of our inability to cultivate the organism *in vitro*) was necessarily lepromatous tissue, this method cannot be considered to be particularly accurate We are at present carrying out experiments with fractional doses to try to estimate the minimum effective dose

In the above series spread of the disease from the site of inoculation as estimated by the finding of organisms in the related lymphatic glands did not occur before the third week following inoculation There is definite evidence of cellular proliferation and phagocytosis of bacilli at the site as early as the eighth

TABLE I  
Showing the general blood picture of epidemic dropsy

Number	Age	Sex	Race	Days from noticing the symptoms	Erythrocytes	Hæmoglobin per cent	Colour index	Leucocytes	PERCENTAGE			
									Polymorph	Small mono	Large mono	Eosinophyl
1	40	Male	Hindu	15	4,000,000	60	75	8,500	69	20	3	8
2	30	Do	Do	18	4,000,000	55	7	11,000	66	22	3	9
3	45	Do	Do	12	3,200,000	50	78	8,000	60	21	8	11
4	52	Fem	Moh		3,940,000	35	44	12,000	56	25	4	15
5	16	Male	I Ch	7	2,100,000	14	33	6,700	60	25	8	7
6	21	Do	Hindu	25	1,500,000	15	5	7,000	70	14	7	9
7	29	Fem	Do	20	3,100,000	32	51	9,430	59	17	5	19
8	18	Male	Do	1	5,000,000	60	6	6,000	74	18	3	5
9	50	Do	I Ch	7	3,200,000	40	62	12,000	74	9	5	12
10	42	Do	Hindu	10	3,700,000	40	54	9,000	60	24	6	10
11	29	Do	Moh	12	3,550,000	55	75	11,000	51	37	8	4
12	12	Do	Hindu	20	4,000,000	60	75	15,000	79	9	4	8
13	10	Fem	Do	1	4,500,000	55	61	8,000	72	20	3	5
14	19	Male	Moh	15	4,000,000	50	62	11,000	70	17	8	5
15	26	Do	I Ch	11	3,500,000	40	57	7,000	67	17	3	13
16	15	Do	Hindu	18	4,000,000	60	75	6,000	69	16	3	12
17	25	Do	Chinese	20	2,000,000	20	5	11 000	80	16	3	1
18	30	Do	Hindu	12	3,500,000	40	57	9,000	72	15	3	10
19	35	Do	Do	16	4,900,000	65	65	10,000	52	34	7	7
20	40	Do	Do	7	3,800,000	55	7	5,600	62	30	6	2

As to the leucocytes the table shows that in most of the cases there is a more or less distinct leucocytosis. Though according to the text-books the normal leucocyte count is 5,000 to 10,000 per c mm, in Bengalees 5,000 to 6,000 per c mm is the usual finding.

For the differential count, the smears were stained with Giemsa's azur-eosin. The table shows that there is an increase of the eosinophyls. In this connection, it is to be mentioned that there is not only an increase of typical eosinophyls having bilobed nuclei and a coarse, eosin-stained granular protoplasm with deficient cell walls, but some of the polymorphonuclears also, instead of having

fine eosinophyl granules, have very coarse, red-stained granular protoplasm. Another peculiarity that will be observed in the differential count, is that in some of the cases there is an increase of the large mononuclear cells. Whether this increase is a peculiarity of epidemic dropsy or is due to its associated intestinal trouble is difficult to say.

TABLE II

*Hæmatocrit readings showing the relative volume of plasma and corpuscles*

	Plasma	Corpuscles
1	70	30
2	57	43
3	64	36
4	60	40
5	65	35
6	68	32
7	68	32
8	70	30
9	57	43
10	65	35

The hæmatocrit reading in Table II shows the relation of the volume of corpuscles to that of the plasma. Blood samples were taken from the vein and oxalated with a weighed amount of the potassium salt and then centrifugalised in a hæmatocrit tube. The relation of the volume of corpuscles to that of the plasma in a normal healthy adult is 48 to 52, but in this condition, it is evident from the table that there is a marked variation. The plasma volume is much increased in relation to that of the corpuscles, and thus there is a distinct hydræmic condition of the blood.

TABLE III

*Showing the coagulation time of blood in cases of epidemic dropsy*

	Sex.	Age	Coagulation time	Method used
1	M	18	3 min 20 secs	Wright's Coagulometer
2	F	26	3 " 30 "	" "
3	M	58	3 " 3 "	" "
4	M	20	4 " 0 "	Capillary Method
5	M	37	4 " 10 "	" "
6	M	16	3 " 20 "	" "
7	M	14	2 " 50 "	" "
8	M	45	3 " 10 "	" "
9	F	23	3 " 20 "	" "
10	M	29	3 " 50 "	" "

The coagulation time of the blood has been noted in some of these cases. The blood is obtained by a finger prick and allowed to be drawn in by capillary action into a fine capillary tube and the time noted. A sharp and bold prick allows the blood to flow freely and it takes about one to two seconds to fill a fine capillary tube of uniform bore of about 6 seconds length. Every 15 seconds a portion of the tube is broken to note the appearance of the fibrin threads. In the normal condition, the usual time taken is 3 to 4 minutes.

The findings in these cases is given in the Table III from which it will be seen that there is not much variation from the normal.

day following inoculation One of us (J M H) has lately performed a series of biopsies in rats at definite intervals after subcutaneous inoculation microscopic sections prepared therefrom bring out the above point

In the 43 positive rats bacilli were recovered from the following areas —

- (1) Site of inoculation in 35
- (2) Associated inguinal glands in 24
- (3) Prevertebral glands in 33
- (4) Spleen in 27
- (5) Liver in 13

From the twenty-eighth week onwards involvement of the lungs in addition to the structures already mentioned has usually been found One notable feature is the apparent immunity of the kidneys and suprarenals bacilli can be demonstrated with relative frequency in the *capsules*, but we have not with certainty recovered organisms from the *substance* of either organ The apparent relative infrequency of involvement of the inguinal glands draining the inoculation site is explained by the fact that extensive ulceration frequently develops at the latter point in advanced cases, leading to a lack of definition of the structures in the neighbourhood There has been no increase of virulence as estimated by shortening of the incubation period, i.e., there is no evidence of increased virulence by *passage*

## II *Intraperitoneal inoculation of rats on a full diet*

The total number of rats on a full diet inoculated intraperitoneally was 48 Of these, 40 were subjected to post-mortem examination and lesions were demonstrated in 37 The negative rats died 2, 3 and 26 weeks, respectively, following inoculation The *known* percentage of positive rats is, therefore, 92.5 and the shortest period after inoculation in which lesions were observed was one week The relationship between the interval which had elapsed since the inoculation and the extent of leprous involvement, slight as it was in the subcutaneous group, has proved in the intraperitoneal series to be even less in evidence—in one case, indeed, in which the animal had survived for 26 weeks following inoculation, careful macroscopic and microscopic examination failed to reveal any evidence of infection

The occasional occurrence of granulomatous tumour-like masses in the abdomen following intraperitoneal inoculations has already been described by us in a separate paper (Muir, Henderson and Landeman) (7)

In the 37 positive rats, bacilli were recovered from the following sites in the undermentioned order of frequency

- (1) Prevertebral glands in 36
- (2) Spleen in 34
- (3) Liver in 34

Involvement of the prevertebral glands and spleen is a relatively early phenomenon, and bacilli have been recovered from both sites as early as one week following inoculation The liver is involved rather later and, while we have been



# THE HYDROGEN-ION CONTENT OF VACCINE LYMPH AND ITS RELATION TO THE POTENCY AND KEEPING PROPERTIES OF LYMPH PREPARED UNDER TROPICAL CONDITIONS \*

BY

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POTENCY and resistance to adverse conditions are the two most essential factors in a vaccine lymph destined for use in a warm climate. Unfortunately, lymphs prepared under tropical conditions show very considerable differences in these two respects without any obvious reasons. The present investigation was undertaken at the King Institute, Gundy, with a view to estimating the influence of the Hydrogen-ion content of the lymph upon these two factors and to laying down the optimum conditions for preparation and storage under tropical conditions. The investigation has consisted of two series of experiments. In the first the reaction of a large number of batches of lymph has been estimated under varying conditions (a) Fresh, (b) After exposure to adverse conditions, and (c) After purification by means of chloroform. These results have thus been correlated as far as possible with the results of vaccinations on children and reduction in the numbers of the bacteria contained in the lymph.

In the second series a suitable lymph was selected and a portion artificially adjusted to different reactions after which the results on calves were noted after different periods of storage.

The glycerinated lymphs used for the experiments were those in routine use in the Institute at that time. Each batch of lymph was the product of 5 or 6 calves so that the readings represent the average rather than the results obtained from individual calves.

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\* These experiments were carried out in 1923 while the authors were respectively Director and Assistant Director of the King Institute, Madras

able to demonstrate bacilli in this organ as early as the first and second weeks after inoculation, this is by no means a constant finding in our experience and it is much commoner to find it involved from about the seventeenth week onwards. The apparent immunity of the kidneys and suprarenals was again noted.

### III *Subcutaneous inoculation of rats on deficient diets*

The total number of rats on deficient diets inoculated subcutaneously with rat leprosy material was 33. Of these 15 were on a Vitamin A-free diet, 4 on a Vitamin B-free diet, while 14 were on a diet of stale fish and rice. 31 rats were subjected to post-mortem examination in all of which lesions were found, and the minimum period after inoculation in which positive findings were recorded was 3 weeks. Bacilli were recovered from the following sites —

- (1) Site of inoculation in 18
- (2) Inguinal glands in 13
- (3) Prevertebral glands in 17
- (4) Spleen in 17
- (5) Liver in 3

### IV *Intraperitoneal inoculation of rats on deficient diets*

34 rats on deficient diets were inoculated intraperitoneally with rat leprosy material. 15 were on a Vitamin A-free diet, 5 on a Vitamin B-free diet, while 14 were on a diet of stale fish and rice. A total of 25 out of the 34 rats were subjected to post-mortem examination and lesions were found in all. The minimum 'incubation period' after inoculation was 2 weeks. Bacilli were recovered from the following sites —

- (1) Prevertebral glands in 22
- (2) Spleen in 23
- (3) Liver in 4

We do not think from the above experiments that there is evidence that deficient diets have any effect in accelerating the onset or in modifying the severity of rat leprosy. It is true that the percentage of *known* positives was in each case 100 but the number of post-mortems performed in each of the experiments was disproportionately low, partly owing to the barium poisoning already consumed by its confreres. In connection with Experiment IV, one point of interest referred to and partly owing to the fact that in these experiments a dead rat was very readily consumed is the apparent infrequency of involvement of the liver. In these experiments as in others of a similar nature to be detailed later we desire to express our indebtedness to Lieut-Col R. McCarrison, Director, Deficiency Diseases Inquiry, Pasteur Institute, Coonoor, South India, for hints on the composition of the various diets.

### V *Subcutaneous inoculation of rats on a full diet with 1|10th usual dose of rat leprosy emulsion*

Nine rats on a full diet were inoculated subcutaneously with 1|10th the usual dose of rat leprosy material. All were subsequently proved on post-mortem examination to have developed lesions. The shortest period after inoculation in

The pulp in each case was diluted to a strength of 1—5 with a 50 per cent solution of glycerine and water whose pH had been carefully adjusted beforehand to 7.2\*

The reaction of the lymph was obtained by estimating its pH by the colimetric method

Two methods of obtaining a clear fluid so that the tests could be read were adopted (a) Method of dialysis used for obtaining the pH of blood, and (b) Centrifugalisation

In the former 0.5 c.c. of lymph, made as above, was put into a freshly prepared collodion sac and floated in 5 c.c. of normal saline solution, whose reaction had been adjusted to 7.0. Preliminary experiment showed that the reaction of the mixture did not give a constant reading until after 4 hours' emersion in the saline. Once the maximum reading had been obtained, however, it remained constant for about 18 hours. The tests were, therefore, set up in the late afternoon, kept in the cold store (10°—12°C.) overnight and the pH of the dialysate estimated early next morning.

In the latter method (centrifugalisation) 0.5 c.c. of lymph were diluted with 2 c.c. of neutral saline (pH 7.0) and centrifuged at high speed for 30 minutes. With lymphs on the acid range the sedimentation was complete and a perfectly clear fluid resulted. When the fluid was slightly alkaline, however, there was a slight haziness which did not, however, interfere with the reading of the tests.

The results obtained by the two methods agreed very closely with each other.

Dialysis was used for the first series of experiments and the centrifugalisation method for the second series.

Before the actual experiments were carried out, the two methods were subjected to a preliminary test to estimate the margin of error due to the technique alone. Two separate experiments were carried out for this purpose.

In the first 1 c.c. of the same lymph (sample I) was added to each of 12 centrifuge tubes and diluted with 5 c.c. of carefully neutralised saline. Each sample was centrifuged and its reaction estimated. At the same time 12 samples of the saline used for the dilution were also tested. This procedure was repeated with a second batch of lymph (sample II) and at the same time half-a-dozen samples of this lymph were dialysed after the manner described above and the reactions of the dialysate tested. Table I gives the results.

In the first lymph the reactions obtained after centrifugalisation showed a variation of 0.2 and in the second the whole 12 samples gave identical readings. By the dialysis method there was a maximum variation of 0.1.

A potentiometer test of lymph No. II carried out at the same time gave a reading of 7.18. The amount of variation due to the methods employed can, therefore, be accepted as not exceeding 0.2 and the readings given in the following experiments can be accepted subject to this proviso.

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\* This slight degree of alkalinity due to the diluent must be allowed for in the readings of the first series of experiments.

which lesions were observed was 12 weeks and bacilli were recovered from the following sites in the undermentioned order of frequency

- (1) Site of inoculation in 9
- (2) Inguinal glands in 9
- (3) Prevertebral glands in 9
- (4) Spleen in 7
- (5) Liver in 3

#### VI *Subcutaneous inoculation of rats on deficient diets with 1|10th usual dose of rat leprosy emulsion*

Nine rats on deficient diets were inoculated subcutaneously in the right groin with 1|10th usual dose of rat leprosy material of these 5 were on a Vitamin A-free diet, while 4 were on a diet of stale fish and rice 8 were subjected to post-mortem examination, and in all lesions were found The shortest period after inoculation in which positive findings were recorded was 9 weeks and bacilli were recovered from the following sites —

- (1) Site of inoculation in 7
- (2) Inguinal glands in 8
- (3) Prevertebral glands in 7
- (4) Spleen in 4
- (5) Liver in nil

It is concluded from these two small experiments that 1|10th the usual dose of rat leprosy emulsion inoculated subcutaneously is capable of producing lesions and that deficiencies in diet do not play an important part in the onset and course of the disease Moreover, there was no absolute relationship between extent of leprous involvement of tissues and strength of the bacillary inoculum in rats sacrificed at approximately equal dates from the commencement of the experiment

#### VII *Intraperitoneal inoculation of rats on a full diet with 1|10th usual dose of rat leprosy emulsion*

The total number of rats on full diet inoculated intraperitoneally with 1|10th the usual dose of rat leprosy material was 9 Of these 8 were subjected to post-mortem examination and in all lesions were found the minimum period after inoculation in which positive findings were recorded was 12 weeks, and bacilli were recovered from the undermentioned sites

- (1) Prevertebral glands in 5
- (2) Spleen in 7
- (3) Liver in 3

#### VIII *Intraperitoneal inoculation of rats on deficient diets with 1|10th usual dose of rat leprosy emulsion*

Nine rats on deficient diets were inoculated by the intraperitoneal route with 1|10th the usual dose of rat leprosy material 6 animals were subjected to post-mortem examination and all were positive 5 were on a Vitamin A-free diet and 4 on a diet of stale fish and rice The minimum period after inoculation

Table II gives the reactions of 35 different cups of lymph immediately after preparation. Results obtained with each lymph on children are also shown by means of the insertion success rate. The case success rate was cent per cent in every instance. The reactions show a considerable range on the alkaline side but the majority lie between 7.4 and 7.8. The lymphs with a reaction not exceeding 7.8 appear also to have given the best vaccination results.

The lymph with a pH of 8.0 may possibly have suffered from a slight excess of alkalinity but the results obtained in later experiment (Table V) do not altogether support this inference.

TABLE I

*Showing the results of a series of preliminary tests with the same samples of lymph to test the accuracy of the technique of the centrifugalisation and dialysis methods*

Method	Substance tested	Samples giving pH of				
		7.0	7.1	7.2	7.3	7.4
Centrifugalisation	NaCl	3	5	4		
	Lymph I	3	1	7		
Centrifugalisation	NaCl	4	8			
	Lymph II				12	
Dialysis	Lymph II				2	4

The pH of lymph II tested by the potentiometer was 7.18

TABLE II

*Showing the reactions of 35 different batches of lymph after preparation together with the insertion success rate obtained on children*

Insertion success rate of lymphs	Number of lymphs giving a pH of											TOTAL.
	7 0	7 1	7 2	7 3	7 4	7 5	7 6	7 7	7 8	7 9	8 0	
100												
95-99			1	1	4	3	1	6	5	1	2	24
90-94							2		1		1	4
85-89											2	2
80-84											2	2
75-79					1							1
70-74									1			1
TOTAL								1				1
			1	1	5	3	3	7	7	1	7	35

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in which positive findings were recorded was 13 weeks and bacilli were recovered from the following sites —

- (1) Prevertebral glands in 5
- (2) Spleen in 6
- (3) Liver in 3

Conclusions similar to those in the parallel 'subcutaneous' series are suggested from these two small experiments, viz, that 1/10th the usual dose of rat leprosy emulsion inoculated intraperitoneally is capable of producing lesions and that deficiencies in diet have no appreciable influence on the onset and course of the disease. The absence of relationship between extent of leprosy, involvement of tissues and strength of original inoculation is again noted.

#### IX *Feeding of rats on rat leprosy material*

A total of 30 rats was fed on rat leprosy material. Of these 20 were on a normal well balanced diet while the remaining 10 were on a diet deficient in Vitamin A. The number of feeds varied from three to nine and the duration of the experiment from 12 days in the former case to ten weeks in the latter. 24 of the 30 rats were subjected to post-mortem examination, and of this number 3 proved positive after periods of 21, 31 and 51 weeks, respectively, following the last feed. All 3 animals were on a full well balanced diet and each had had a total of three feeds only. Bacilli were recovered from the following sites —

- (1) Spleen in 2
- (2) Lower prevertebral glands in 2
- (3) Mesenteric glands in 1
- (4) Axillary glands in 1
- (5) Liver in 1

Of the rats put on a Vitamin A-free diet, several survived till the twentieth week from the last feed but no lesions were detected. This diet does not therefore appear to favour the more rapid incidence of lesions after feeding experiments.

Marchoux and Sorel(2) also succeeded in producing lesions by feeding with massive doses. They state that this route of administration may give rise to a primary lung infection without involving the cervical or mesenteric glands.

It is suggested that repeated feedings of healthy rats with heavily infected rat leprosy tissue may give rise in a small proportion of cases and after an incubation period of at least twenty weeks to definite lepromatous lesions, but the fact that all three positive rats had had a total of three feeds only while others which had been subjected to a larger number of feeds (up to a maximum of nine) were consistently negative, urges caution in interpreting the results of this experiment. It is possible, though rather unlikely(6), that all these animals were suffering from a naturally acquired rat leprosy infection. The experiment at least demonstrates that the results obtained by the administration of rat leprosy bacilli orally are very irregular.

Twenty of the lymphs tested above were retested after varying periods of exposure to room temperature during the Madras hot weather (average maximum temperature about 98.4°F). In this table an attempt has been made to record the three factors concerned (a) The duration of the exposure in days (denoted by the figures given in the body of the table), (b) The change in reaction between the first and second tests, and (c) The percentage loss in the insertion success

TABLE III

*Showing the change in the pH of 20 lymphs exposed to room temperature (average 98.4°F) for a varying number of days together with the percentage loss in the insertion success rate in each case*

Change in pH after exposure		Percentage loss in insertion success rate											TOTAL No
		0	10	20	30	40	50	60	70	80	90	100	
Alkaline	0.8												2
	0.6	5											
	0.4			8									
	0.2							6	7				
No Change						65					88		11
			8										
	0.2	4								10	10	10	
Acid			6	6	5								7
	0.4	4		5									
			4										
	0.6										8		
	0.8												
TOTAL													20

The numbers in the body of the table refer to the days the lymph has been exposed. Each number refers to a separate batch of lymph.

rate. Eleven of the lymphs show changes in reaction not exceeding 0.2 in either direction. These changes are within the limits of experimental error and may therefore be neglected. Of the remaining 9 samples, however, 7 have deviated towards the acid side and only 2 towards the alkaline. It would thus appear that there is a definite tendency for a lymph when exposed to become more acid. Unfortunately the effect of this acid formation on the vaccination rates cannot be gauged for other factors, especially the exposure to heat, have been more potent in causing degenerative changes in the lymphs and have thus obscured other possible relationships.

It should be noted that the reduction in the numbers of living bacteria in the exposed lymphs was enormous (between 100 and 99.7 per cent in every case).

X *Scarification of young rats with rat leprosy material*

Ten young rats (from one to three days old) were scarified on the abdominal wall with rat leprosy material. 9 of the animals were subsequently examined post-mortem and of these 3, which had survived for 40 weeks, 43 weeks and 56 weeks, respectively, following scarification were found to have developed signs of the disease. The negative rats were post-mortemed 20 weeks (3), 24 weeks (1), 29 weeks (1) and 30 weeks (1) after scarification. Bacilli were recovered from the undermentioned sites —

- (1) Site of scarification in 3
- (2) Inguinal glands in 3
- (3) Spleen in 3
- (4) Axillary glands in 3
- (5) Prevertebral glands in 2
- (6) Mesenteric glands in 2
- (7) Liver in 2
- (8) Lungs in 2
- (9) Omentum in 1

In a tributary experiment in which scarification was combined with other modes of infection (*viz.*, subcutaneous or intraperitoneal inoculation) the minimum period within which lesions developed was 12 weeks.

It is concluded that scarification of young rats may be followed after a long interval by the development of definite leprosy lesions.

XI *Inoculation of human leprosy material into rats*

Twenty-three rats were inoculated with human leprosy material rich in bacilli, in 19 of these leprosy emulsion alone was employed, in 2, leprosy emulsion plus an equal quantity of whole blood from the bacillary donor and in the remaining 2, leprosy emulsion plus an equal amount of donor's serum. 19 animals were on a full well balanced diet, 4 on a diet deficient in Vitamin A. Post-mortem examinations were performed on 19 out of the 23 rats and bacilli were recovered from sites at a distance from the seat of inoculation in 3. In all the positive animals the bacilli had been introduced by the intraperitoneal route. 2 were on a full diet, one on a Vitamin A deficient diet. The periods which had elapsed since inoculation were respectively 6 weeks, 15 weeks and 20 weeks and bacilli were recovered from the following sites —

- (1) Prevertebral glands in 3
- (2) Spleen in 1
- (3) Left inguinal gland in 1

The number of bacilli recovered from each of the above mentioned sites was small and in no case was there any evidence of a generalised lepromatous condition such as one finds after inoculation of the rat leprosy organism into these animals.

In two cases the animals survived up to a period of 78 weeks from the date of inoculation but in them no acid-fast organisms were recovered. Inoculations of portions of tissues taken from the positive rats into other 2 rats gave negative results.



The same tendency to become acid is seen in lymphs which have been subjected to chloroform vapour for purpose of bacterial purification (Table IV). The lymphs have been tested after 2½, 5, 7½ and 10 minutes' exposure to the vapour. Out of the 10 lymphs so tested the final pH in 4 did not differ more than 0.2 from the primary reaction in the 2. The reaction was more alkaline and in 4 it was more acid. During the 2½ and 5 minute periods the swing towards the acid range is especially noticeable. The changes appear to be very similar to those observed in the lymphs which were exposed to room temperature but have occurred in a very much shorter time. In both cases the reduction in the living bacteria was very considerable. It is thus a question whether the acid formation may not be due to the bacterial destruction. The evidence brought forward at present, however, is insufficient to prove that this is the cause.

The second series of experiments was undertaken on different lines. A single batch of lymph which was known to be potent was taken and its reaction was artificially adjusted to a pH of 4.6, 8 and 10.

Portions of it were then stored for 4 weeks at varying conditions of temperature, i.e., 55°F and 98.4°F (room temperature) and tested periodically for potency.

Children were not used for the tests in this case as it was thought that there would be too many failures to justify this procedure. The routine vesiculation rate test\* on calves was utilised instead. The dilutions of the lymph used in each case were 1—10 and 1—100. These are shown in the tables as numerators and denominators.

The results of this experiment are given in Table V.

The samples adjusted to pH 4.0 and pH 10.0 have given negative results under all conditions showing that these extreme ranges of acidity and alkalinity have rapidly destroyed the vaccine virus.

The sample with a slightly acid reaction (pH 6.0) has given some results up to the third week when stored at 55°F but at the higher temperature, the virus has deteriorated rapidly and died.

The sample adjusted to pH 8.0 has given the best results under all conditions. It shows little or no difference with the control (pH 7.4 to 7.5) when stored at 55°F and is definitely more resistant to adverse conditions of temperature.

In every case the percentage reduction in the numbers of bacteria was very high (Table VI).

These experiments show that the vaccine virus is very susceptible to even slight degrees of acid whereas it is much more tolerant and in fact survives best, under adverse conditions, when the reaction is definitely alkaline.

According to our first series of experiments, however, the majority of lymphs show a definite swing towards the acid range when subjected to storage or to adverse conditions such as heat.

Unless used absolutely fresh we consider that our investigation shows, therefore, that all samples of lymph should have their reaction carefully adjusted to a point which is very definitely alkaline, say pH 7.6 to 7.8.

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\* The number of vesicles per inch obtained with a given dilution of the lymph

It is concluded that after inoculation of human lepra bacilli by the intraperitoneal route organisms can occasionally be recovered from the prevertebral glands, the inguinal glands and the spleen. This occurrence is, however, relatively rare and in no case does anything in the nature of a generalised lepromatous infection occur.

XII *To test the possible effect of Calmette's B C G culture in protecting against subsequent infection with rat leprosy*

- Twenty rats on full diet were inoculated with Calmette's B C G culture followed by inoculations (subcutaneous and intraperitoneal) of rat leprosy bacilli at intervals of from 12 days to 3 weeks thereafter. 10 animals were subjected to post-mortem examination and all showed evidence of infection, lesions being discovered in one case as early as the second week following inoculation. 3 of the positive animals had been inoculated with rat leprosy suspension subcutaneously, the remaining 7 intraperitoneally. The frequency of involvement of the different sites was the same as in the control group.

It is concluded that Calmette's B C G culture has no effect in protecting against infection when rats are subsequently inoculated with rat leprosy by the subcutaneous or intraperitoneal route.

XIII *The effect of inoculation of human and rat leprosy on the Chinese hamster*

Ten hamsters on full diet were inoculated subcutaneously with human leprosy and 8 with rat leprosy (the latter by both subcutaneous and intraperitoneal routes). 7 animals from the former group including 3 which survived till the sixteenth week following inoculation were subjected to post-mortem; all were negative. The same result was found in an examination of 7 animals from the latter group including 2 which survived till the fourteenth week.

It is concluded that the Chinese hamster is not highly susceptible to either human or rat leprosy and that recognisable lesions do not develop before at least the sixteenth week in the case of the former or before the fourteenth week in the case of the latter.

XIV *The effect of heat on rat leprosy bacilli*

Tissue rich in rat leprosy bacilli was heated at 60°C for periods of from 5 to 25 minutes and then inoculated into ten rats. All the animals with one exception were killed between the twenty-fourth and twenty-seventh weeks; lesions were found in all except those inoculated with material heated to 60°C for 25 minutes. Contrasted with the controls, there were remarkably few lesions in rats which had been inoculated with heated material considering the length of time that had elapsed between the dates of inoculation and death; however, there was no correspondence between duration of heating and extent of lesions.

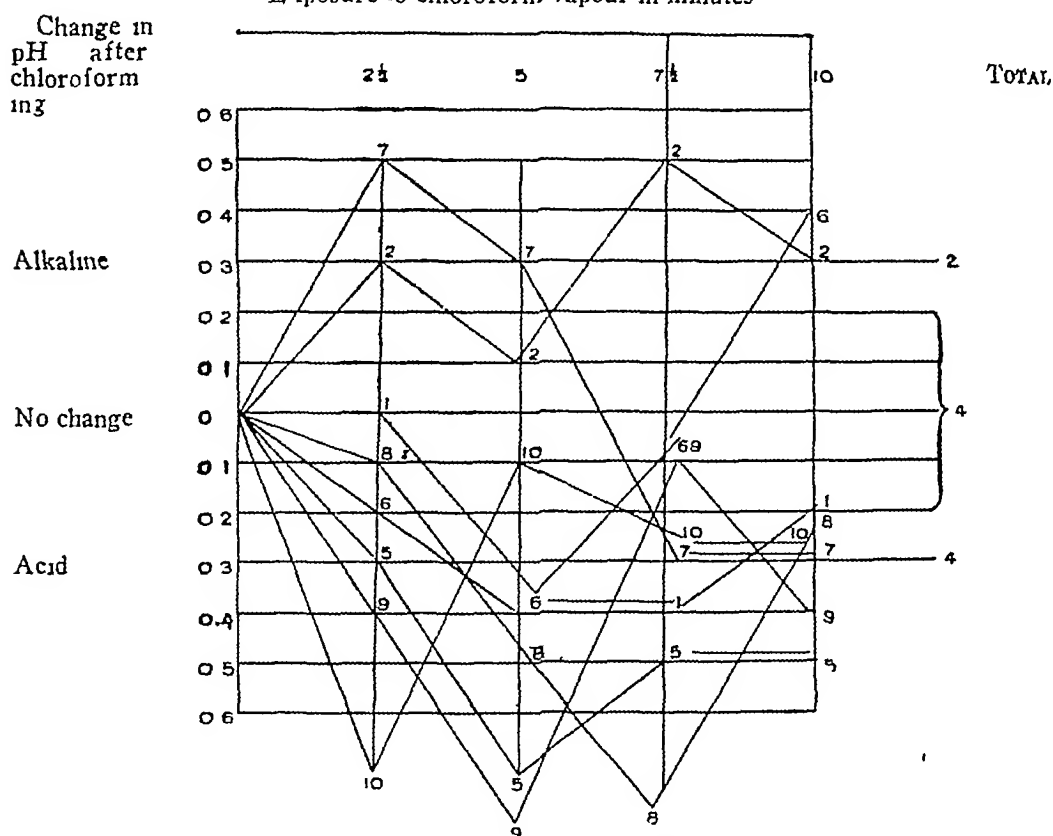
It is concluded that exposure to a temperature of 60°C for 25 minutes or longer is lethal to rat leprosy bacilli. Marchoux and Sorel(2) found that the rat leprosy bacillus when exposed to a temperature of 60°C will survive for fifteen minutes.

In this way the possibility of deterioration owing to the formation of acid during storage or exposure would be effectually removed

TABLE IV

Showing the change in the pH of 10 lymphs after exposure to chloroform vapour for varying periods of time

Exposure to chloroform vapour in minutes



In this chart the same numbers refer to different tests on the same lymph

#### SUMMARY

1 The chemical reaction of vaccine lymph prepared and stored under tropical conditions has been investigated. Glycerinated lymph was used for the tests.

2 Dialysis and centrifugalisation were used to obtain a clear fluid for test purposes.

3 In 35 freshly prepared batches of lymph the pH varied between 7.2 and 8.0, the majority lying between 7.4 and 7.8.

After exposure to room temperature (98.4°F) for varying periods, the majority of lymphs became more acid. There was at the same time a great reduction in the numbers of bacteria.

4 The same tendency to an increased acidity was seen after the lymphs were treated with chloroform vapour for purification purposes.

5 When the reaction of different samples of the same batch of lymph were artificially adjusted to pH 4, 6, 8 and 10 and then stored at 55°F and room temperature (average 98.4°F) for different periods of time, it was found that the sample whose reaction was adjusted to pH 8.0 gave the best results.

XV *The effect of preservation of rat leprosy bacilli outside the body*

Portions of tissue containing numerous rat leprosy bacilli were preserved for varying periods of time (varying from 24 hours to 8 days) outside the body at room temperature prior to their inoculation by the subcutaneous and intraperitoneal routes into 14 rats. All the animals died or were killed at periods between  $17\frac{1}{2}$  and  $25\frac{1}{2}$  weeks and in all lesions were found again there was no precise relationship between length of time of preservation and extent of lesions subsequently developed.

XVI *Inoculation of Japanese dancing mice with human leprosy bacilli*

Nine Japanese dancing mice were inoculated with human leprosy emulsion by the intravenous, intraperitoneal and subcutaneous routes. This was an attempt to repeat the work of Sugai(9) who claims to have produced spreading lesions in 18 out of 24 such animals. Post-mortem examination was performed on 6 of the mice, but all were negative including 2 which had survived for periods of  $17\frac{1}{2}$  and 18 weeks following inoculation.

In addition to the above mentioned investigations the following miscellaneous experiments were also carried out.

XVII Six rabbits were injected with rat leprosy intravenously and intraperitoneally. One animal survived for 19 weeks following inoculation and in this as in the 4 others examined no acid-fast bacilli were found.

XVIII Three rats were given subcutaneous and intraperitoneal inoculation of a one per cent solution of trypan blue for histological purposes.

XIX Six rats were inoculated (3 subcutaneously and 3 intraperitoneally) with material from a suspected case of rat leprosy in a human being. Of these 5 were subjected to post-mortem examination. Death of the animals occurred at varying intervals between 2 days and 27 weeks following inoculation but no acid-fast bacilli were recovered.

XX Two white rats were injected with rat spleen material from an animal which had previously been inoculated with human leprosy. Both animals were killed after 25 weeks but no acid-fast bacilli were recovered.

XXI On account of the striking results obtained lately (Muir)(8) by the use of potassium iodide in human leprosy, a small experiment was performed to test the effect of this drug on rats infected with the rat form of the disease. 2 rats were given potassium iodide daily for 14 days. No febrile reactions comparable to those in the human form of the disease occurred. The experiment was small and incomplete and should be repeated, in particular a much more accurate estimate of dosage relative to body weight is required.

XXII In co-operation with Major R. B. Lloyd, I.M.S., the Imperial Serologist, the Wassermann reaction was performed on a series of 34 rats, of which 16 were healthy and 18 highly leprous. The reaction was consistently negative in all.

*The following experiments are at present in progress*

(a) The effect of half the normal dose of rat leprosy suspension on rats kept on a diet of stale fish and rice. The suspension has been inoculated both by the subcutaneous and by the intraperitoneal routes.

TABLE V

Showing the keeping properties of samples of the same batch of lymph artificially adjusted to different reactions and exposed for four weeks to 55°F. and room temperature average (94.8°F)

Temperature of exposure		Originally adjusted to	pH AT END OF				Dilution	VESICULATION RATE ON CALVES AT END OF			
			1st week	2nd week	3rd week	4th week		1st week	2nd week	3rd week	4th week
55°F	Expt	4	4	4.5	4.5	4.5	1-10	0	0	0	0
							1-1000	0	0	0	0
		6	7.6	6	6	6	etc	Cont	0.19	3.95	0
								0	0	0.78	0
		8	8	8	8	8	etc	Cont	4.5	4.7	2.26
								1.3	0.19	0.18	0
		10	10	9	10	9.5	etc	0	0	0	0
								0	0	0	0
		Control	7.5	7.5	7.4	7.4		Cont	Cont	4.26	4.0
								1	0.2	0.18	0
Room temp 94.8°F	Expt	4	4	4	4	4.5	etc	0	0	0	0
								0	0	0	0
		6	6	6	6	6	etc	2.2	0	0	0
								0	0	0	0
		8	8	8	8	8	etc	Cont	1.01	0	0
								0.7	0.2	0	0
		10	10	9	10	9.5	etc	0	0	0	0
								0	0	0	0
		Control	7.5	7.5	7.5	7.3	etc	2.1	0	0	0
								0	0	0	0

6 The investigation shows that there is a tendency in most lymphs in the tropics to become more acid on storage or exposure, and, as the formation of acid has a deleterious action on the vaccine virus, we recommend that the reaction of each batch of lymph should be carefully adjusted to a point which is definitely alkaline, say pH 7.6 to 7.8, to guard against deterioration from this cause.

We desire to express our indebtedness to the assistance given in this work by Mr K. V. Sundaram, M.Sc. (Calcutta), Chemical Assistant, and by Khan Sahib Mohamed Omar Sahib, First Vaccine Assistant at the King Institute of Preventive Medicine, Guindy, where the work was carried out.

(b) The effect of simultaneous inoculation of rat leprosy suspension and of serum from a rabbit which had previously been repeatedly inoculated with rat leprosy bacilli intravenously

(c) Philibert(10) suggests that the mouse is likely to prove suitable for inoculation of bacilli of human origin since it can be infected artificially with the bacillus of Stefansky and yet does not suffer naturally from the latter organism, being thus free from the sources of fallacy to which the sewer rat is liable. A series of English mice has been inoculated by the subcutaneous, intraperitoneal and intravenous routes with a suspension of human leprosy bacilli, in order to elucidate this point

(d) In order to estimate, if possible, the minimum effective dose of rat leprosy bacilli, 10 rats on a full diet have been inoculated subcutaneously with 1/100 the usual dose of rat leprosy suspension as estimated by the opacity tube method

(e) Tissue rich in rat leprosy bacilli has been kept at cold room temperature (approximately 36° to 44°F) for 24 hours prior to subcutaneous inoculation into 3 white rats

(f) Attempts are being made to cultivate the rat leprosy bacillus *in vitro*. Various media have been used, both liquid and solid and attempts have been made to grow the organism at room temperature and at incubator temperature under both aerobic and anaerobic conditions. So far all efforts have been negative. acid-fast organisms have been found on certain media as late as 4 months after implantation of the original piece of rat leprosy tissue but until now all attempts at subculture have proved negative. We, therefore, conclude that the presence of the bacilli as noted above represents a mere process of survival

(g) In connection with various experiments on monkeys we had occasion to inoculate rat leprosy material both by the subcutaneous and by the intraperitoneal routes into 3 of those animals. all of them died at intervals of from 8 to 16 weeks after inoculation with signs of marked generalised tuberculosis. The presence of this infection was confirmed by animal inoculation into guinea-pigs. This experiment is being repeated

(h) The series of changes which occur in the skin of the rat following subcutaneous inoculation of rat leprosy material is being studied. Portions of skin are removed by biopsy at definite intervals after inoculation. interesting results are being obtained which may possibly throw light on the pathology of the naturally acquired human infection. It is hoped in due course to make this investigation the subject of a separate paper

### Summary and Conclusions

(1) Rats can be infected with rat leprosy by the scarification, subcutaneous and intraperitoneal routes of inoculation. In connection with the two last mentioned routes, spread of the infection has been demonstrated as early as about the third week following inoculation, while microscopically evidence of cellular proliferation at the site of a subcutaneous inoculation has been detected as early as the eighth day thereafter

TABLE VI  
Showing the percentage reduction in the numbers of bacteria per c cm of lymph in samples of lymph stored in the previous table (Table V)

PERCENTAGE REDUCTION					SAMPLES EXPOSED TO 55°F EXPERIMENT II				SAMPLES EXPOSED (AVERAGE 94.8°F) EXPERIMENT III			
Originally adjusted to	PH AT THE END OF				After storage for				After storage for			
	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week	1st week	2nd week	3rd week	4th week
4	4	4.5	4.5	4.5 to 5	100	98.7	99.7	99.3	97.0	98.7	99.7	100
6	6	6	6	6	100	100	99.7	95.4	99.0	99.3	98.4	100
8	8	8	8	8	97.7	99.7	99.3	99.0	99.3	100	98.4	98.0
10	10	10	10	9 to 9.5	99.3	99.3	98.7	99.0	100	99.0	99.3	100
Control	7.5	7.5	7.5	7.2 to 7.4	99.3	99.3	99.0	99.0	99.7	99.0	99.3	100

Original specimen gave a count of 7 600 per c cm

Original specimen gave a count of 7 000 per c cm

(2) The infrequency of lesions within the kidneys and the suprarenals has been commented on and the occasional production of granulomatous tumour-like masses after the intraperitoneal route of inoculation has been noted

(3) Increased virulence of infection does not occur in rats fed on Vitamin-deficient diets, or on a diet rich in protein-decomposition products

(4) Lesions can be produced in a small number of cases after feeding rats with infected tissue but the incubation period is a long one and the results are capricious

(5) No lesions comparable to those seen in the rat have been obtained in the monkey, the rabbit or the Chinese hamster after the inoculation of rat leprosy material

(6) Rat leprosy bacilli are rendered non-virulent by exposure to a temperature of 60°C for 25 minutes or longer

(7) Inoculation of human leprosy material into rats, Chinese hamsters and Japanese dancing mice gave completely negative results

(8) Calmette's B C G culture has no effect in protecting rats against subsequent infection with rat leprosy bacilli

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THE METHOD OF FEEDING OF *PHLEBOTOMUS*  
*ARGENTIPES* WITH RELATION TO ITS BEARING  
ON THE TRANSMISSION OF KALA-AZAR

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THE account given below of the method of feeding *Phlebotomus argentipes* is not an attempt to give an exact and detailed description of the physical mechanism of feeding in this insect, as this would entail a study of the sum total of the actions and interactions of the various tissues, muscle, nerve, chitin, etc., involved in the process, a study we have had no time or opportunity to make. Rather, it is an attempt to give a general description of the act of feeding, with special reference to its bearing on the possibility of transmission of kala-azar by this insect.

In studying this question two methods have been employed by us (a) observation of the living fly in the act of feeding, and (b) the study of microscopical sections of the fly fixed *in situ* in the act of feeding, and with its proboscis embedded in the skin. The results of these observations will be considered separately below.

THE LIVING FLY IN THE ACT OF FEEDING

*Technique*—For easier observation the flies were fed in tubes on experimental animals instead of in muslin cages as is usually done. The animal, mouse, or rabbit, was tied to a board and its abdomen shaved clean. The flies were then liberated directly on to the feeding surface by inverting the tubes containing them on to the skin. Once the flies had commenced to feed, the tubes



could be removed for better observation. Observation was by the naked eye, hand lens, microtelescope, or binocular microscope according to the magnification necessary. It was found an advantage to employ flies ready for a second or subsequent feed, as they feed more readily under such conditions than those having their first meal, and for certain observations, to use, in preference, flies which during oviposition had got their wings moistened with water resulting in their sticking together. In such a case the wings are stuck together above the body of the fly and so do not obscure the entry of blood into the alimentary canal.

### THE METHOD OF FEEDING

71

*Selection of a site for feeding*—If the fly is going to feed one finds that as soon as it is liberated on the skin it begins to quest, with a short jerky progress, all over the surface within the limits of the containing tube, giving the impression that it is searching for a suitable place at which to feed. That this is probably the case seems to receive corroboration from the fact that the fly here and there makes tentative punctures, sometimes continuing to feed at the first site selected, but often withdrawing its proboscis at once and making trial of several more sites before one is finally found to its satisfaction.

*Position when feeding*—As the fly thrusts its proboscis into the selected site on the skin it makes its position as secure as possible by spreading its legs widely on all sides, the result of this manoeuvre being to bring the body of the fly much closer to the skin on which it is feeding. At the same time, owing to the fact that in feeding the head and proboscis of the fly make an angle of at least  $90^\circ$  with the thorax, the body of the fly lies practically parallel to the surface of the skin. The wings, in feeding, are always spread out horizontally over the body and legs. This attitude, characteristic of feeding, with wings and legs widely spread, and body nearly flattened on the skin, is the very antithesis of the normal appearance of the fly. It gives the impression of a limp and exhausted insect with sagging limbs which cannot properly support it, while the normal appearance is one of extreme alertness as though the fly were on 'tip toes'.

The antennæ, as a rule, are held well up in the air as though being intentionally kept out of the way of the parts occupied in feeding. They make a comparatively small angle between them and, although straight, have a strong resemblance to the horns of a black-buck.

The maxillary palps appear to lie on the skin laterally, making a very wide angle between them. They may even lie at right angles to the line of the body and usually present a slight curvature with its convexity anteriorly. The piercing organs are actually thrust into the skin to a depth considerably exceeding half the length of the proboscis and, owing to the comparative shortness of the latter, the head appears to be very closely applied to the surface of the skin. The position and shape of the labium in feeding are very characteristic. The two apical segments are spread out upon the skin at an angle as wide as their attachments will allow, and one cannot help comparing them

## BIOCHEMICAL OBSERVATIONS ON THE BLOOD

The total nitrogen of the blood has been estimated by Kjeldahl's process 1 cc of oxalated blood being taken in every case for the incineration with Merck's pure sulphuric acid The catalyser used was copper sulphate

The protein content of the blood was calculated from the estimated total nitrogen, deducting the value of the non-protein nitrogen from that of the total nitrogen

TABLE IV

*Showing the non-protein nitrogen and the protein content of the blood and serum in cases of epidemic dropsy—in grammes per 100 c c of blood*

Number	Total nitrogen of blood	Non-protein nitrogen	Blood proteins	Total nitrogen of serum	Serum protein
1	1.4	1.21	8.6		
2	2.24	0.35	13.7	1.4	7.5
3	2.52	0.283	15.37		
4	2.19	0.28	13.57		
5	2.38	0.35	12.79	1.2	7.25
6	2.47	0.42	15.17	9.94	5.9
7	1.54			1.12	
8	1.61	0.35	7.96	9.38	5.64
9	2.52	0.28	17.57	1.05	6.37
10	2.52	0.28	17.57	1.12	6.81
11	2.24				
12	2.1	0.246	12.7	1.05	6.37
13	2.3	0.302	13.68	1.12	6.81
14	1.8	0.236	10.6	9.66	5.89
15	2.32	0.336	14.2	1.34	8.12
16	2.19	0.224	13.5	9.94	6.07
17	2.37	0.28	14.62	1.12	6.81
18	2.45	0.286	15.2		
19	1.93			9.61	
20	2.2	0.36	13.51	1.05	6.31
21	1.4	0.25	8.56	9.38	5.7
22	2.1	0.24	12.93	9.52	5.8

From the results of the estimations it will be found that the total nitrogen and consequently, the protein value are much below the normal. In Europeans, the normal total nitrogen varies from 3 to 3.2 per cent and in Bengalees, as has been found by the writer, it is from 2.8 to 3 per cent. In this condition, the maximum figure obtained is 2.52 per cent and the minimum 1.4 per cent.

The total nitrogen of the serum has also been estimated by the above process. Though in some cases, the value has gone down, in most cases, it is near about the normal. This fact shows that in spite of the anæmia and hydræmia the serum proteins retain the normal value and also it is evident that it is the diminished number of the erythrocytes that is mainly responsible for the diminished total nitrogen value.

The non-protein nitrogen content of the blood was estimated in the same way as the total nitrogen from the filtrate obtained after precipitation of the proteins with 5 per cent trichloroacetic acid. In normal conditions, the value of the non-protein nitrogen varies from 0.2 to 0.42 per cent and it will be seen from the findings that they keep fairly to the normal in these cases. The usual pathological condition in which the nitrogenous metabolites, i.e., the non-protein nitrogen, are retained, in the blood, is when the functional efficiency of the kidneys is impaired. In epidemic dropsy the renal function remains intact. In the course of this investigation numerous samples of urine have been examined and nothing has been found to suggest renal involvement. The only abnormality that can be detected in many of the samples is the marked reaction for indican. This is, of course, as might be expected, since intestinal trouble is a common feature of the disease.

The urea content of the blood has been estimated by Marshall's process with urease. The urease used in these estimations has been isolated by the writer from the seeds of a very common bean found in Bengal, known as 'makham sheem'. The enzyme activity of the isolated urease has been found to be even better than that obtained from the soya-bean. From the findings given in the Table V, it will be seen that there is not much variation from the normal values.

The 'creatinine' content of the blood has been estimated by the Folin's method, and the figures obtained do not show any pathological deviation.

The only abnormality suggesting retention of nitrogenous metabolites is the high figure obtained in the uric acid values. They were estimated by Benedict's modification of Folin's method. The normal uric acid content of the blood, as is found in text-books, is 2 to 3 mgs per 100 c.c. of blood. The usual diet of Bengalees, as compared with Europeans, is relatively very poor in purin bodies. One would therefore naturally expect rather lower values in the uric acid content, but it is rather peculiar that much higher values are obtained even in severer cases. In these cases the absence of renal involvement, the relatively poor purin diet and the absence of any data suggesting an extensive breakdown of the nuclear material of the body cells, makes it very difficult to decide if the figures obtained really represent uric acid. Whether it is due to any fault in the technique or to the presence of something producing colour in the reagent similar to that of the uric acid, is very difficult to say. In some

to large flat feet taking as firm a stance as possible. They form the two sides of a wide angle between which the piercing structures of the proboscis enter the skin.

The labium, at the junction of its upper third and lower two-thirds, is bent posteriorly in the middle line to at least a right angle, but, when the proboscis is fully thrust in, the angle is an acute one and in some cases the upper and the lower parts of the inner surface of the labium may be closely approximated to one another. It is quite evident that one of the functions of the labium, with its firmly planted apical segments, is to steady the delicate piercing organs and to support them during the more violent movements performed in the process of feeding. The labium appears also to have a tactile function as flies, before feeding, are often seen to feel the surface of the skin with the labium as though selecting a suitable site to pierce.

*The entry of blood into the alimentary canal*—As soon as the flow of blood commences it can be seen through the lateral walls of the thorax, which are almost entirely naked, as a band of pink colouration extending towards the abdomen. This colouration can be seen in the thorax as long as feeding is continued. As the blood reaches the abdominal part of the alimentary canal it can be seen gradually to fill the midgut from before backwards. In appearance it is of a much darker colour at first than the pink colouration seen in the thorax. As the filling of the midgut extends backwards the walls of the gut become greatly distended to contain the blood meal until the whole abdomen appears bloated and of the colour of a red currant. This change from the darker colour of the earlier stages of feeding is undoubtedly due to the stretching of the abdominal parietes, which renders them more transparent and allows the blood to shine through more distinctly. As soon as feeding is completed the fly rapidly withdraws its proboscis in one movement. If it is now examined it will be found that all the blood is contained within the abdominal portion of the midgut and that the thoracic portion is clear.

The whole time occupied in feeding, from the commencement of the flow of blood into the thorax to the full repletion of the fly, is comparatively short and may vary between two and five minutes. On the other hand it sometimes happens that the fly may remain for a long period with its proboscis thrust into the skin before there is any sign of the flow of blood into the fly. Once this commences, however, the process seems to be completed without further delay.

Flies are sometimes found to imbibe, not blood, but a pale yellow liquid which distends the abdomen in a similar manner to an ordinary blood meal. This appears to be a serous fluid obtained from the wound and the cause of it is not known as such flies show no abnormality in the proboscis such as might prevent the ingress of red blood cells.

It has always been considered that during, and immediately after, the act of feeding there is never any voiding of fæces by the sandfly, such as occurs in the case of fleas and some other insects, this act not being performed, as a rule, until two or three days after the blood meal. This conception we have found to be quite erroneous. A fly in the act of feeding can be conveniently

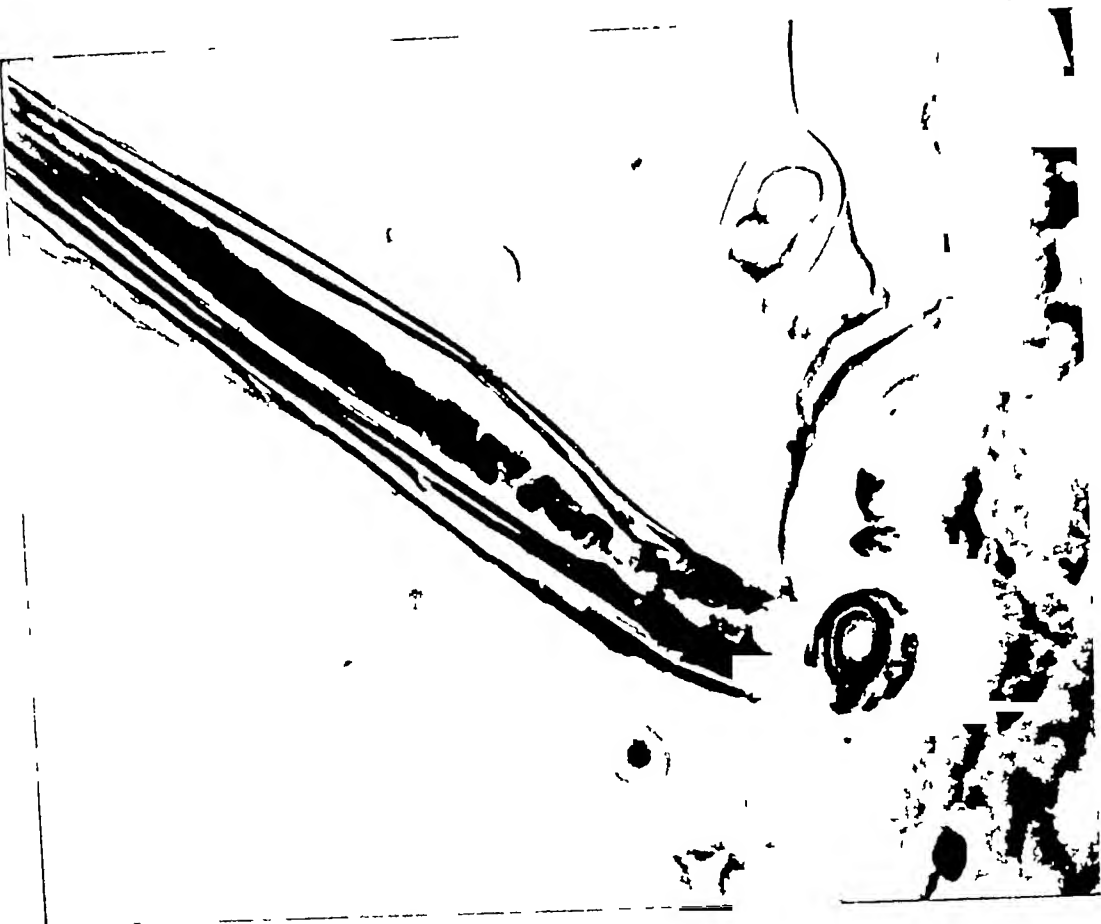


Fig 2

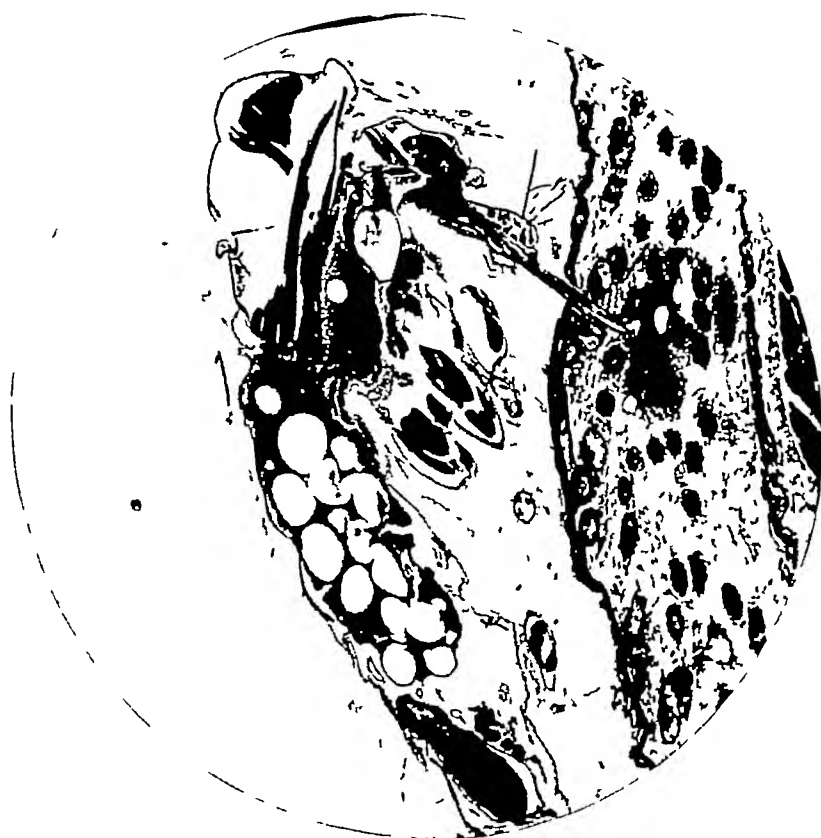


Fig 1

observed with a sufficient degree of magnification by means of a binocular microscope if the tube be detached from the stand and used in the hands. The course of events is somewhat as follows —

Soon after feeding has commenced, and after some blood has entered the anterior end of the abdomen, the anal orifice of the fly is seen to distend and a drop of opalescent fluid appears and is discharged. As feeding continues, and the blood fills the abdomen, many more droplets of fluid are, at frequent intervals, ejected from the anal orifice. The droplets subsequent to the first are clearer than the latter and present only a slight opalescence. They are ejected with such considerable force that they come to lie on the skin of the experimental animal at a distance of from one and a half to twice the entire length of the fly. Here they form minute raised droplets on the skin showing little tendency to spread out. During the course of feeding as many as fifteen or more such droplets may be ejected. As the fly sometimes inserts its proboscis in more than one place before it is completely fed, it necessarily changes its orientation in the different areas it occupies and it would be quite easy for some of the droplets to contaminate the wounds made by the proboscis or even to be discharged directly on to them. As there is usually no development of *L. donovani* in the hindgut of the fly it seems unlikely that the habit of the fly here being discussed can have any bearing on the transmission of this parasite. The only way in which this might possibly be effected would be for the forms of *L. donovani* present in the midgut of the fly—and such forms are always present—gradually to be pushed posteriorly by the advancing blood and eventually ejected in some of the droplets of fluid. Our attempts to obtain stained preparations from the fluid of the droplets have never resulted in the demonstration of any forms of *L. donovani*.

#### OBSERVATIONS FROM SECTIONS OF FLIES FIXED IN SITU IN THE ACT OF FEEDING

*Technique*—The object aimed at was to obtain preparations in which the fly, actually in the act of feeding on an animal, was instantly killed and fixed in situ with its proboscis embedded in the skin. From such preparations microscopical sections, through fly and skin combined, were prepared. For convenience in handling, as well as for economy, small animals are to be preferred, and we always utilised white mice.

The description given below indicates the essential steps in the technique in their proper sequence and, where necessary, explanations of the various steps are given.

1 The mouse is tied on its back to a small board by its four legs, and the abdomen is shaved, using water only without soap.

2 A test tube containing the fly is inverted on the abdomen of the animal.

3 As soon as blood is seen (by use of a hand lens) to be entering the fly there is dropped on to the latter, by means of a long capillary pipette provided





with a rubber teat, one drop of the following fixative, which should be freshly prepared

Absolute alcohol	}	Equal Parts
Chloroform		
Glacial acetic acid		
Mercury perchloride to saturation		

The fixative should be dropped directly on to the fly, the end of the pipette being steadied by the left hand, from a height of not more than one centimetre. If not dropped directly on the fly the latter will withdraw its proboscis even though it be killed.

4 Without any delay one delivers on to the fly from another pipette a drop of fresh egg albumen mixed with not more than its own volume of distilled water, the mixture being prepared by shaking with beads.

5 Still without any loss of time, the mouse on its board is slowly completely immersed in a vessel of freshly prepared Bles fluid

70 per cent alcohol	90 c c
40 per cent formaldehyde	7 c c
Glacial acetic acid	3 c c

This immersion should be carried out in a deep vessel containing enough of the fluid to allow of complete immersion of board and mouse.

With a razor two deep transverse gashes into the body of the mouse are made about half an inch above and below the site occupied by the fly. This serves a threefold purpose (a) It immediately kills the mouse (b) By stopping the local circulation, it prevents the carriage away by the blood of any parasites inoculated by the insect should it be one capable of causing disease by its bite (c) The immersion causes coagulation of the albumen and so firmly fixes the fly in its place. In this latter respect the previous fixative would be preferable and is only replaced by the Bles fluid on the score of economy since a large bulk is needed.

6 A large piece of the whole thickness of the abdominal wall of the mouse, with the fly in its centre, is removed to a smaller vessel of Bles fluid and, by means of fine scissors and forceps, a very small piece containing the fly is cut out and put into a small vial of Bles fluid. Previous to this the fly may, if so desired, be still more firmly attached to the skin by allowing a few drops of the albumen mixture to fall on it through about one centimetre depth of the fixative.

7 The tissue, with fly attached, is kept in the fixative for 12—24 hours. It is then brought into 70 per cent alcohol and through graded alcohols to absolute alcohol.



8 It is then passed into clove oil until clear, when it is transferred to celloidin dissolved in clove oil. In this it remains a minimum of three days but may be kept for some weeks.

9 It is then subjected to the process of double embedding in celloidin and paraffin.

10 For the most intelligible results, longitudinal sections should be cut.

*Note*—The essentials for the fixative used to drop on the fly are that it should instantaneously kill it as well as act as a fixative. The former purpose is best served by a fixative which causes immediate and complete wetting. For this purpose the Bles fluid, which contains no absolute alcohol or chloroform, is not suitable. Certain other fixatives such as Carnoy's fluid may be used, but the one recommended has been found the best, and has the additional advantage that the mercury present causes a very rapid coagulation of the egg albumen subsequently put on the fly.

*Description of a median sagittal section through fly and skin.* *Position of fly*—The body of the fly lies approximately parallel to the surface of the skin (Plate LXIX, fig. 1).

*Depth of insertion of piercing organs*—These are inserted to a depth somewhat greater than half the length of the proboscis. The actual depth of insertion is greater than that shown in the microphotographs because in the process of fixation the tissues of the skin undergo a certain amount of shrinkage, a defect to which the rigid chitinous structures of the proboscis are not subject (Plate LXIX, fig. 1).

*Depth of tissue penetrated*—It will be seen from the illustrations that the epidermal layer of the skin is of fairly uniform thickness except where the hair follicles extend into the deeper areolar tissue. The fully inserted piercing organs may extend into the tissues to a depth of at least six times the thickness of the epidermal layer. This degree of penetration enables them to reach the situation only of the smaller blood vessels as the larger vessels are chiefly situated at a depth greater than that reached by the bases of the hair follicles. For the same reason the superficial muscular layer of the skin, as a rule, is not penetrated. At the same time, in certain cases, where the skin has been tightly stretched, we have obtained sections showing the tip of the proboscis almost reaching the muscular layer.

*Method of obtaining blood*—In certain cases we have seen the piercing organs actually to enter small blood vessels but we do not consider that, as a rule, the blood is sucked directly from such vessels. We believe that what usually happens is that the damage done by the tips of the stylets to the capillaries and small blood vessels causes an extravasation of blood into the loose areolar tissue reached by them, and that this extravasated blood is then sucked up by the fly. In a live animal the evidence of this extravasation of blood remains on the skin in the form of a small reddened area which may persist for some hours. The condition described is well seen in Plate LXIX, fig. 1. The apparent bubbles in the extravasated blood are, in reality, fat cells, as a glance at the unaffected surroundings of the area will show.



*Flow of blood*—The enclosed canal through which the blood flows up the proboscis has throughout its length a diameter which will allow the passage of at least three red cells abreast even in its narrowest part. This is well shown in Plate LXIX, fig 2 which represents the distal half of the proboscis. From the mouth proper of the insect to the distal termination of the pharynx, the same comparatively narrow channel persists and this is true also of the buccal cavity. In the pharynx itself a different condition is found. Here the channel commencing narrowly at its distal end expands and is widely distended, at its bulbous proximal end, and a very thick column of blood can be accommodated as will be seen from Plate LXX, fig 3. The membranous œsophagus, also, although normally narrow, has a considerable capacity for distension and a thick column of blood can flow through it into the midgut. The midgut itself can, of course, be enormously distended.

*Position of parts other than the piercing parts*—This has already been dealt with in the section on the living fly, and the only part which appears in a median sagittal section is the labium which has already been adequately dealt with. Its position and shape during feeding are shown in Plate LXX, fig 4.

*Structure of the canal through which blood passes up the proboscis*—Nitzulescu (1926) has stated that Christophers, Shortt and Barraud (1926) in their description of the anatomy of the head of *P. argentipes* describe the canal through which the blood traverses the proboscis as being composed of the hypopharynx below and the labrum-epipharynx above, and he observes that this is not really the case. He maintains that the canal is formed by the overlapping mandibles inferiorly and the labrum-epipharynx superiorly. This, we believe, is also the description given by Grassi (1907) in the case of *P. papatasi* of the position of the mandibles when at rest. In this connection we should point out that Nitzulescu appears to have misunderstood to some extent the description and figures in the publication referred to [Christophers, Shortt and Barraud (1926)]. In this account the description and figures apply only to the portion of the alimentary canal in the region of the prestomum and not to the blood channel in the proboscis itself as seems to have been inferred by Nitzulescu. In order to settle this point, since the position of the mandibles at rest might not be the same as their position during feeding, it would be necessary to examine flies fixed in the act of feeding and while blood was actually passing up the proboscis. This we have been successful in doing, and examination of cross sections of the proboscis gives a clear solution of the problem.

In such preparations the presence of the blood cells indicates clearly where the channel lies and by what structures it is bounded. If sections made near the distal end of the proboscis are examined it will be seen that the blood lies in a channel formed inferiorly by the mandibles, almost completely overlapping one another, and superiorly by the labrum-epipharynx (Plate LXXI, fig 5). If serial sections are now examined progressively in the direction of the proximal end of the proboscis it will be found that, as one passes up the proboscis, the mandibles overlap one another less and less until they cease to form an unbroken wall for the inferior boundary of the blood channel (Plate LXXI, fig 6).

#### EXPLANATION OF PLATE LXX

- Fig 3 Approximately longitudinal section showing bulbous proximal  
end of pharynx containing a very thick column of blood  
„ 4 Section showing position of labium in the act of feeding

Eventually the mandibles are widely separated from one another and the inferior wall of the blood channel is definitely formed by the hypopharynx (Plate LXXII fig 7). A consideration of the lateral origin of the mandibles from the mandibular condyles will make it evident that the construction of the blood channel described is the natural result of the anatomical relations of the various parts concerned. So far as our observations in a limited number of flies go, the point where the mandibles cease to form unaided the inferior boundary of the blood channel is situated near a point where a transverse plane would pass through the junction of the maxillary palps with the maxillæ. The actual point of separation of the mandibles may vary according to the position in which they have been fixed in the individual fly, but the variation is within the comparatively short range of  $30\ \mu$ . Owing to the longitudinal curvature and narrowing at the base of the mandibles the transition from a complete overlapping of these structures to their separation is comparatively abrupt and takes place within a distance of  $50\ \mu$  in the longitudinal axis of the proboscis.

#### BEARING OF THE PRECEDING DESCRIPTION OF THE METHOD OF FEEDING OF *P. argentipes* ON THE TRANSMISSION OF KALA-AZAR

Until actual transmission of kala-azar by the bite of *P. argentipes* has been obtained any discussion as to the *modus operandi* by which the result is achieved must necessarily be confined to the domain of theory. With this proviso however, our actual sum of knowledge as regards the conditions of a heavily infected fly comprises certain known facts which would appear to justify us in drawing certain tentative conclusions as to the manner in which the infection is conveyed to the mammalian host.

As the narrowest parts of the alimentary canal of the sandfly, viz., the parts in the proboscis, the buccal cavity and the distal end of the pharynx, will admit of the passage of at least three red blood cells abreast, it follows that a single flagellate could always pass down the channel, should it contain a stationary column of fluid. It is at least doubtful whether such a stationary column of blood ever exists in that part of the alimentary canal contained within the head and proboscis of the fly. It is also doubtful if the flagellate could progress anteriorly were the alimentary canal empty since the channel in a great part of its length would then be collapsed.

The column of fluid in the alimentary canal when the fly begins feeding so far from being stationary probably sets in a strong current in a direction towards the midgut. The tendency of this would be to sweep individual flagellates in a posterior direction since the flagellates are not capable of swimming against a comparatively rapid current. This can readily be proved by watching flagellates in fluid medium in which artificial currents have been set up.

These considerations all tend to lead to the first conclusion we may form viz., that the entry of individual flagellates into the wound caused by the bite of *P. argentipes* is not likely to be an active process due to any volition on the part



- Fig 5 Cross section near the distal end of proboscis showing complete overlapping of mandibles to form inferior wall of food channel Note blood cells in the lumen
- „ 6 Cross section higher up the proboscis at point where the mandibles just fail completely to form inferior wall of food channel Note blood cell lying between the two mandibles

of the flagellates caused by chemiotaxis or any other cause. Its introduction, if it occurs, must be caused by some agency to which the active swimming power of the flagellate affords no appreciable assistance.

What applies to single flagellates in this respect applies with still greater force to groups or plugs of mutually adhering flagellates. Such groups while potentially actively motile as regards their individual units have no power of co-ordinated movement as a whole in any given direction.

In any very heavily infected fly it will be found that the portion of the alimentary canal anterior to the oesophagus is practically blocked with a solid plug of flagellates (Plate LXXII, fig. 8). This blockage, while not always complete in the widest parts of the channel, definitely occludes, for all practical purposes, the narrow portions in the distal end of the pharynx and in the buccal cavity. Any chinks left between the flagellates in the narrower portions of the channel, while they might allow the passage of fluid, would prevent the entry of any considerable number of the solid blood cells.

Conversely, if a column of blood cells had entered the proboscis they would interfere with and probably prevent the expulsion in an anterior direction of a solid plug of flagellates passively expelled by the fly.

The conclusion drawn from these considerations is that in the case of a very heavily infected fly where the anterior parts of the alimentary canal contained in the head are filled with solid plugs of flagellates, the expulsion of these plugs anteriorly, by active muscular efforts on the part of the fly, must precede a successful blood meal. That the dislodgement of the plugs of flagellates must take place anteriorly is necessitated by the fact that the bulk of the flagellate growth is vastly greater as it extends backwards from the buccal cavity and, as it is practically continuous, pressure applied at any part of the channel by muscular action would inevitably tend to expel the contents in an anterior direction where the resistance would be less.

The general conclusion to be drawn, then, would appear to be that if kala-azar be transmitted by the bite of *P. argentipes*, the infective material is likely to be in the form of a solid plug of flagellates and that this plug is introduced into the wound by an active expulsive effort on the part of the sandfly preliminary to the taking of the blood meal.

It was in order to verify these conclusions in a practical manner that flies fixed in situ in the act of feeding were examined by us in microscopical sections. The difficulties in technique are considerable and have resulted in our being able to examine only a few infected flies in this manner, since many of our best preparations proved to be uninfected flies. In one very heavily infected specimen certain bodies were noticed lying in the tissues of the experimental animal at the side of the proboscis near its tip which closely resembled the flagellates present in the mouth parts. As the fallacies possible in identifying one or two individual flagellates in iron-haematoxylin-stained areolar tissue are obvious we do not wish to stress this finding, but we believe that if time were



Fig 5



available to practise and develop this technique some definite and conclusive findings would result. The technique is also one which could be applied to the investigation of other insects conveying parasites by their bite such as mosquitoes.

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EXPLANATION OF PLATE LXIX

- Fig 1 Median sagittal section of *P argentipes* fixed in the act of feeding Note extravasation of blood
- " 2 Longitudinal section of distal end of piercing organs of *P argentipes* showing flow of blood





Fig 7



Fig 8.

TABLE V

*Showing the non-protein nitrogen, urea, 'creatinine' and uric acid contents of blood in cases of epidemic dropsy.*

Number	Sex	Non-protein nitrogen	Urea	'Creatinine.'	Uric acid
1	M	021	026	0012	.
2	M	035	028	0014	0037
3	M	0283			005
4	M	028	020	.	0034
5	M	035	025		00389
6	M	042	028	0013	0028
7	F		022	0014	
8	M	035	027	0014	
9	M	028		.	0047
10	M	028	022	0013	00348
11	M		024	0012	0028
12	M	0246	023		00364
13	M	0302	030	0014	
14	M	0236		0014	
15	M	0336	022	0012	
16	F	0224		0012	
17	M	028		0014	00436
18	M	0286	024		004
19	M		022		
20	M	036	025	0014	0044
21	M	025	018	0013	0044
22	M	024	029	0013	00348

of the cases both Folin's direct method and Benedict's modification have been tried with the result that though there was slight variation in some individual cases, the figures were consistently higher than normal

It has already been noted from the examinations of the urine that marked reaction for indican is a common feature of the disease. In the intestine, by putrefactive decomposition of proteins, phenols and indols are produced. Tyrosin and phenyl alanine yield phenol compounds, while tryptophane yields indole and skatole. These substances in their passage through the body are oxidised and then are paired with sulphuric acid in the liver and are finally excreted in the

urine for the most part as the potassium or sodium salt of these bodies. The indole which is only one of the products of putrefaction comes out in the urine as potassium indoxyl-sulphate or indican. Tyrosine and phenyl alanine give

TABLE VI

*Showing the glucose, chlorides and calcium content of blood in cases of epidemic dropsy*

Number	Sex	Glucose	Chlorides	Calcium
1	M	114	56	00609
2	F	10	502	00957
3	M	10	49	016
4	M	-	508	011
5	M		544	0074
6	M		527	0078
7	M		502	0068
8	M		567	0073
9	F	1	627	0082
10	M	11	59	0074
11	M	105	55	
12	M	125	59	
13	M	135		0082
14	M	118		014
15	M	12	52	014
16	F		49	012
17	M	12		011
18	M	11	532	0082
19	M	12	55	011
20	M	105		0078
21	M		54	015
22	M		508	014
23	M	095	55	013
24	M	10	52	011
25	M	11	58	013
26	M	10		0068
27	M		52	014

EXPLANATION OF PLATE LXXII

- Fig 7 Cross section still higher up the proboscis at point where the mandibles are completely separated and the inferior wall of the food channel is formed by the hypopharynx Note blood cells in lumen
- „ 8 Approximately longitudinal section through heavily infected *P. argentipes*, showing —
- P Proximal bulbous end of pharynx blocked with plug of flagellates
  - O Œsophagus in same condition
  - D Commencement of diverticulum dilated with flagellates
  - M Midgut dilated with massive growth of flagellates.

TABLE VII

*Showing the calcium content of blood in epidemic dropsy and the result of calcium administration in such cases*

Number	Sex	Calcium—grammes per 100 c.c. of blood	Number of days from the appearance of symptoms	Number of days under calcium
1	M	00609	20	0
2	F	00957	15	4
3	M	016	About a month	15
4	M	011	23	8
5	M	0074	10	0
6	M	0078	15	0
7	M	0068	12	2
8	M	0073	About a month	3
9	F	0082	20	0
10	M	0074	15	0
11	M	0082	10	0
12	M	014	15	6
13	M	016	5 weeks	18
14	M	012	10	0
15	M	011	15	4
16	F	0082	23	10
17	M	011	1½ months	15
18	M	0075	11	3
19	M	015	20	8
20	M	014	23	8
21	M	013	15	5
22	M	011	3 weeks	10
23	M	013	5 weeks	15
24	M	0068	3 weeks	0
25	M	014	3 weeks	8

case that had not had calcium in some form or other for some days. The drug became a fashion even amongst the laity. The estimations in these later cases were above normal, but how far this was of help to the patients could not be said.

# REICHERT

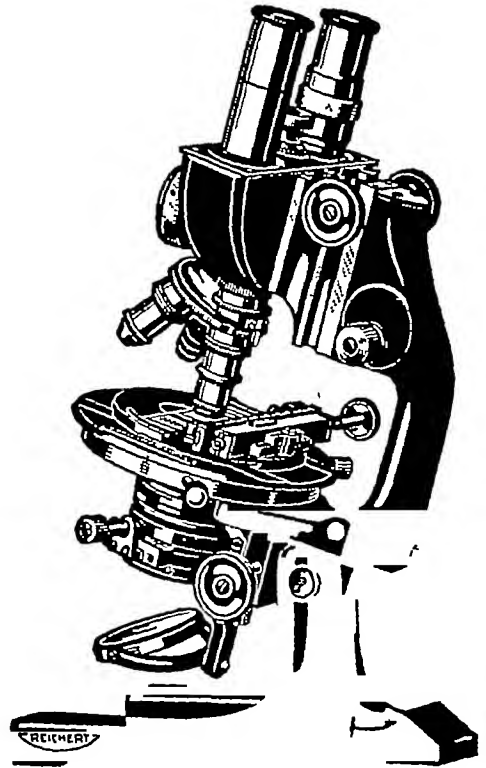
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much decreased and at its expense the globulin content has increased many times. In most of the cases the globulin content is nearly three times the albumin content and in some it is even more.

TABLE IX

*Showing the serum proteins, albumin and globulin content of the blood serum in cases of epidemic dropsy*

Number	Sex	Serum proteins	Albumin	Globulin
1	M	8.66	2.1	6.56
2	M	4.8	1.3	3.5
3	F	6.37	1.5	4.87
4	M	7.42	1.56	5.86
5	M	6.36	1.56	4.8
6	M	6.91	2.1	4.81
7	M	6.18	1.56	4.63
8	M	7.00	1.5	5.5
9	M	5.86	9.62	4.9
10	M	6.56	2.1	4.46
11	M	8.33	4	7.93
12	M	6.02	1.3	4.72
13	M	6.98	1.3	5.68

TABLE X

*Showing the results of analysis of the serum in epidemic dropsy and normal control cases*

The results are given in grammes of nitrogen per 100 c.c. of the serum

Number	Race	Sex	Age	Ser Alb	Ser Glob	Euglob	Pseudo-glob I	Pseudo-glob II
1	M	M	35	32	.8	Trace	7.86	0.14
2	Chinaman	M	43	21	78.1	126	46.4	2.12
3	M	M	45	20.3	9.17	.07	7.56	0.07
4	H	F	44	33.6	7.14	.035	5.39	.14
5	M	M	18	24	.88	Trace	.81	.07
	Christian	M	38	21	.56	.049	4.97	0.14
7	H	M	29	6.58	5.04	.025	3.89	.09
8	M	M	32	5.88	4.76	.04	4.08	.028







rise to phenol and methyl phenol or cresol. Some of these are thrown out with the faeces, the remainder are absorbed. The greater part of the absorbed phenol and cresol is excreted conjugated with sulphuric acid, but a small portion is free in the urine and a part is conjugated with glycuronic acid.

In epidemic dropsy the intestinal trouble leads to the formation of these phenols and their derivatives in large amount and they, being absorbed, are present in the blood. It may be suggested that these phenols give rise to blue colour similar to that of uric acid with the reagent meant for the colorimetric estimation of uric acid. It is quite possible that it is the presence of these bodies that is responsible for the higher figures in the uric acid values.

The glucose content of the blood was estimated by Folin's method. The results do not show any pathological deviation. In Bengalees the normal values are slightly higher than in Europeans, varying from 1 to 12 grms per 100 c.c. of blood.

The chlorides of the blood were estimated by Vollhard's method, and the results show that in most of the cases the content is higher than normal. It is evident that there is retention of chloride. In the absence of any data suggesting renal incapacity, it is rather difficult to explain this retention of chlorides, unless it is suggested that there is imperviousness of the kidney to chlorides. On that assumption it will also be easy to explain the œdema of the epidemic dropsy. The accumulation of sodium chloride in the body may bring about retention of water in the tissues owing to its effect on the osmotic pressure.

The retention of chlorides in these cases with œdema is significant. In the absence of any sign of renal incapacity, and the presence of cardiac trouble which is a common feature of the disease, it has been thought that the œdema is cardiac in origin. The fact that the œdema is present in a marked degree in those cases where the cardiac trouble is least and also the fact that many of the cases have died of heart-failure without any appreciable œdema, throw doubt on the idea that the œdema is purely of cardiac origin. The hæmatocrit readings of the blood in epidemic dropsy show that there is a hydræmic condition of the blood, and the mottled appearance of the œdematous portions, the general redness and sometimes the presence of areas of capillary dilatation with pemphigus-like eruptions show that the œdema is not merely passive and that there is some active pathological change going on in the capillaries and blood vessels, so much so that in some cases the capillaries have actually multiplied leading to the formation of angiomatic growths. It is known that toxic substances which are retained or formed in the body may increase the attractive capacity of blood and especially of the tissues for water (Von Noorden). This idea has been supported by the investigations of Cohnheim and Lichtheim. They have shown that the presence of certain poisons in the blood favours the development of anasarca. These poisons make the walls of the capillaries more pervious and stimulate them to active secretion. In epidemic dropsy it seems that all these factors are at work.

The calcium content of the blood has been estimated by Kramer and Tisdall's method. The results in the untreated cases are decidedly lower than in the normal. It is only at the earlier part of the investigation that the writer could get cases which were not on large doses of calcium, later it was impossible to find a single

(1) *Examination of the blood* For this purpose we use a special type of box with a sliding panel which holds the squirrel in such a way that a drop of blood can be obtained from the tail without any direct handling by the attendant

The box is  $7\frac{1}{2} \times 4\frac{1}{2} \times 7$  inches in size and is made of wood and metal with the exception of one side which is of glass (Plate X, fig 1)

At one end there is a sliding door of the same size and shape as that on the squirrel cages Inside the box is a metal sliding panel curved in its lower part to the degree necessary to form a space just large enough for the squirrel to fit into when the panel is pressed close to the end of the box This panel is operated by a rod through the end of the box opposite to the door and when pushed home can be locked in position by means of a pin outside the box The corners of the box, opposite each end of the little chamber formed by the panel,\* are cut away to the extent necessary and are replaced by two little doors sliding vertically which can be raised when required Each of these has a small notch in its lower edge

The *modus operandi* is as follows —

The box, with the sliding panel withdrawn to the side farthest away from the door, is placed end to end with the squirrel cage, the two doors being placed together Both doors are then raised, the squirrel driven into the box, and the door closed The panel is then moved up carefully, and, when the squirrel takes up the required position across the box, it is driven home and locked

The squirrel is now disposed so that its head is opposite one of the small side doors and its tail opposite the other, nor can it turn round in the confined space of the chamber formed by the panel and the end of the box The small trap door at the tail end of the squirrel is now raised sufficiently to enable the attendant to draw out the tail and is then shut down again, the tail fitting into the notch in the door The specimen of blood can then be taken in the ordinary way

When the film is obtained the panel is released, the squirrel is freed, and is returned to its cage in the same way as it was removed

Throughout the whole operation, which only takes a minute or two, the squirrel, with the exception of its tail, is not handled by the attendant

## 2 *The supply of larger quantities of blood over prolonged periods*

In this case the squirrel has to be caught and held by the attendant A special type of forceps and a small muzzle have been designed to render this operation safe (Plate X, fig 2) The forceps is about 12 inches long and consists of two blades hinged together about their centre The blades can be locked when closed, by means of a ratchet arrangement, similar to that employed on many surgical clamps

The distal ends of the forceps are bent at right-angles to the main axis of the instrument and each blade forms rather more than one half of a circle so that when closed the forceps forms a complete circle with the distal ends overlapping each other The end of each blade is also turned slightly outwards The size of the circle thus made is about 1 inch in diameter and is just large enough to go round the squirrel's neck without compressing it

---

\* The corner on the glass which forms one of the sides must also be cut away

Estimations of cholesterol were done by Bloor's method taking 3 c c samples of oxalated whole blood for each estimation. The results would show that they are markedly lower figures than the normal values. Hypocholestræmia is generally found in those people whose diet is very poor in lipoids, and in the anæmic, and as both these conditions are present in most of the cases examined, no other significance could possibly be attached to the condition found.

TABLE VIII

*Showing the cholesterol content of blood in cases of epidemic dropsy*

Number	Sex	Cholesterol	Nitrogen	Number of erythrocytes per cmm of blood
1	M	130	1.4	3,100,000
2	M	133	2.24	4,000,000
3	M	104	2.52	4,000,000
4	M	111		4,500,000
5	M	144		
6	M	128		
7	M	166		
8	M	144		
9	F	100		4,000,000
10	M	089	2.19	35,000,000
11	M	156	2.47	45,000,000
12	M	104		
13	M	133		
14	M	141		
15	M	185		

#### SERUM PROTEINS

It has already been shown that the total nitrogen of the serum is near about the normal even in spite of the diminished total nitrogen of the whole blood. This total nitrogen represents the proteins and the non-protein nitrogen of the serum. The proteins of the serum are albumin and globulin, and the globulins are further subdivided into three varieties—euglobulin, pseudoglobulin I and pseudoglobulin II. All these have been isolated and separately estimated according to the method described by the writer in another paper (*Indian Medical Gazette*).

In the normal condition the quantity of albumin in the serum is more than the globulin and the relation is approximately as 1.5 is to 1. The findings in Table IX show that there is great deviation from the normal—the albumin content has—

#### EXPLANATION OF PLATE X

- Fig 1 Two boxes showing (*a*) the moving panel half forward with the squirrel about to be caught (*b*) The panel in position with the squirrel's tail outside the box ready for bleeding
- „ 2 (*a*) Showing the special forceps and muzzle alone, and (*b*) the methods of holding the squirrel

Cases 7 and 8 are normal healthy adults

In Table X will be found the relative proportion in which the different globulins are present in these cases. It is evident that the euglobulin and pseudoglobulin II do not take part in the increase and it is the increase of pseudoglobulin I that is responsible for the increase in the total globulins.

My thanks are due to Lieut-Col F A F Barnardo, M A, M D, F R C S, F R C P E, C I E, C B E, I M S, Principal, Medical College, and Lieut-Col A C MacGilchrist, M A, M D, D S C, M R C P, I M S, Professor of Physiology, for giving me all possible facilities both in the Laboratory and in the Hospital in carrying out this investigation.

My thanks are also due to my colleagues for the valuable help I have received from them throughout this work, and specially to Dr Ananta Kumar Dutt, M B, for helping me with cases of epidemic dropsy from the Out-patient's Department.

I desire also to take this opportunity to express my indebtedness to my friend Babu Probodh Kumar Bhattacharyya, B Sc, who helped me materially in carrying out the experimental portion of the work.

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# A SIMPLE METHOD OF HANDLING SQUIRRELS AND OTHER SMALL ANIMALS FOR BLEEDING AND OTHER PURPOSES

BY

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[Received for publication, March 8, 1927]

IN 1923 we first utilised the common Indian squirrel (*Scirus palmaris*) as an experimental animal for our researches with *Spirochaeta carteri*, and found it to be most suitable for this purpose. In the course of our experiments it was found, however, that the continuous handling of infected animals was a source of some danger owing to the quickness of their movements and sharpness of their teeth, for no fewer than six cases of accidental infection, which were directly due to bites of infected squirrels, occurred amongst the laboratory staff during the earlier period of our investigation.

This danger constituted a decided drawback to the use of an otherwise perfect animal, and I therefore devised methods of handling squirrels which have had the effect of reducing the risk of infection to a minimum.

As other investigators in India have begun to use this animal for experimental purposes and, as the methods are also applicable to other small animals, the following account of my technique may be of use.

Infected squirrels are kept in separate cages,  $10 \times 5 \times 8$  inches, made of wood, with strong wire on the two sides and a sliding door at one end.

In the Relapsing Fever Inquiry the squirrels are used to keep alive the spirochaete strains by direct passage, from one animal to another and to act as the source of the living spirochaetes used for the serological tests which have, up to the present, formed the basis of the Inquiry.

For these purposes it is necessary to make both a simple examination of the blood to ascertain the presence of the spirochaetes and their numbers, and to take larger amounts of blood, in some cases over a prolonged period of time.

Each requirement calls for a separate technique.



TABLE I

*Showing the effect of immersing 'street virus' brains in ether for periods varying from 24 to 144 hours*

ETHERISED BRAINS "			INOCULATION				Cerebrospinal fluid.	Negri bodies	REMARKS	SUBPASSAGE			REMARKS
No. of hours immersed	No of brains treated	Sample taken from	No of rabbits inoculated	Deaths	No of days to					No of rabbits subpassaged	No of days to		
					First symp-toms	Death					First-symp toms	Death	
24	1	Outside Inside Mixture	1 1 1	1 1 1	11 16 15	15 18 17	St St St	++ ++ +	Rabies Rabies Rabies				
48	1	Outside Inside Mixture	1 1 1	 1 1	 18 20	 20 22	 St St	 + +	Alive 156 d.ys afterwards Rabies Rabies				
72	1	Outside Inside Mixture	1 1 1	 1 1	 Suddenlv  107	 13  107	St  St	+  —	Alive 156 days afterwards Rabies  —	  1	  28	  Died from diarrhoea	
84	1	Outside Inside Mixture	1 1 1	1 1  	12 22  	13 25  	St Con  	— —  	Rabies? Rabies? Alive 155 days afterwards	1 1 1	 3 13	Alive 155 days afterwards Not rabies Without symp toms Rabies	
96	1	Outside Inside Mixture	1 1 1	 1 1	 Suddenlv  	 34  128	St  St	—  —	Alive 169 days afterwards  This rabbit showed definite tremor 8 days after inoculation but recovered and later died without symptoms No subpassage done	1  1	  11	Without symptom Negri bodies—	
120	1	Outside Inside Mixture	1 1 1	1 1  	   	2 96  	St St  	  —	No subpassage No subpassage Alive 180 days afterwards	  	  	  	
144		Outside Inside Mixture	1 1 1	   	   	   	   	   	Alive 183 days afterwards Alive 183 days afterwards Alive 183 days afterwards	   	   	   	

St = Sterile

Con = Contaminated

The blades of the forceps distal to the hinge, are slightly curved along their length, the concavity facing in the same direction as the ring at the end of the forceps

The muzzle (Plate X, fig 2) is made from perforated metal (brass) roughly to the shape of the squirrel's head, and has two catches, one on the ventral and the other on the dorsal aspects of the opening for the neck of the squirrel. The dorsal catch is hinged and is operated by a small handle running along the dorsal aspect of the muzzle so that the catch can be moved out of the way when the muzzle is being put on. When released a spring returns the catch to its original position and keeps it there.

The catches fit on to the dorsal and ventral aspects of the ring on the forceps and keep the muzzle in position.

The forceps and muzzle are used as follows —

The squirrel is driven from its cage into a wide-mouthed jar and is stupefied with ether. It is then grasped by the skin of the posterior part of the back by a rat forceps and removed from the jar. The squirrel forceps is closed round its neck, fixing its head. The rat forceps is then removed. The attendant can now grasp the animal round the body together with the forceps—its back lying along the slightly curved blades which prevent undue extension of the spine. Finally, the muzzle is put on, the ventral catch being hooked on first (Plate X, fig 2).

The squirrel is now rendered safe and can be held in this position for any length of time, the muzzle guarding against any possibility of accident due to a lapse on the part of the attendant.

The operations just described take at the most two or three minutes for their execution.

#### SUMMARY

A box, forceps and muzzle are described by the use of which squirrels or other small biting animals can be held for bleeding and other purposes, with perfect safety to the attendant.

TABLE II

*us' brains to ether vapour for periods varying between 24 and 120 hours*

No of hours immersed in cerebro-spinal fluid	Negri bodies	REMARKS	SUBPASSAGE			REMARKS
			Number of rabbits sub-passaged	Number of days		
				First symptoms	Death	
	+	Rabies				
	+	Rabies				
	+	Rabies				
12		Great loss of weight (520 grms in 31 days)	1		15	Loss of weight 150 grms in 15 days Subpassage from this rabbit Paralysis 28 days Death 36 days Further subpassage Death 82 days, Negri bodies—
		Not rabies, no subpassage Alive 252 days afterwards				
84	+	Alive 252 days afterwards				
	—	Rabies	1	9	13	Rabies
		Rabies				
96		Great loss of weight	1	8	12	Rabies
		Rabies	1			Alive 242 days afterwards
		Rapid emaciation	1		8	Great loss of weight (630 grms in 8 days) Subpassage from this rabbit Alive 205 days afterwards
n	—	Subpassage not done	1		116	Died of diarrhoea
	—		1			Alive 161 days afterwards
120		Great loss of weight (166 grms in 29 days) Alive 251 days afterwards	1	20 paresis	97	Negri bodies— Subpassage Died from diarrhoea in 11 days
		Alive 251 days afterwards				
		Died without symptoms	1		33	Died without symptoms Subpassage from this rabbit Alive 169 days afterwards
			1			
144		Not rabies, no subpassage done			54	Died of diarrhoea Negri bodies—

St = Sterile

Con = Contaminated

PLATE X

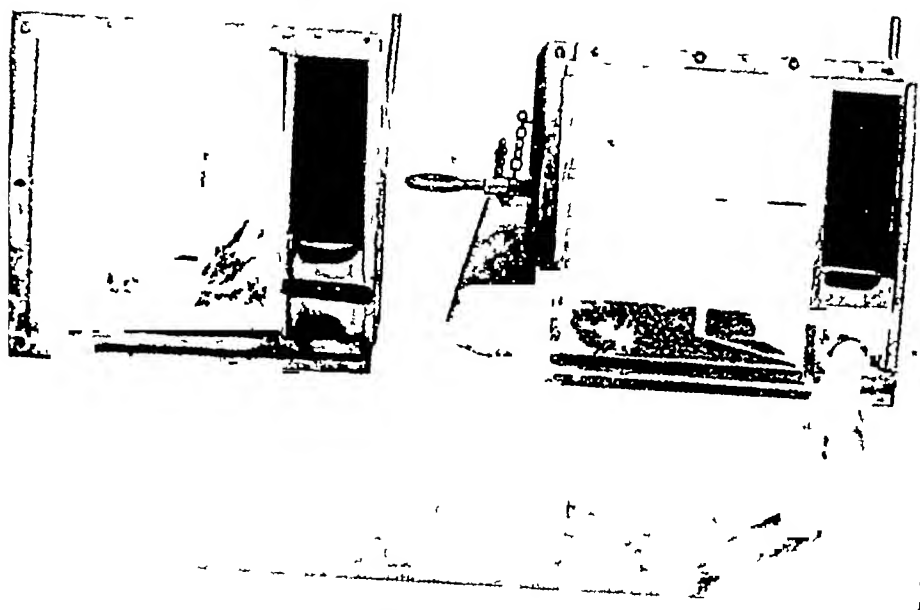


Fig 1

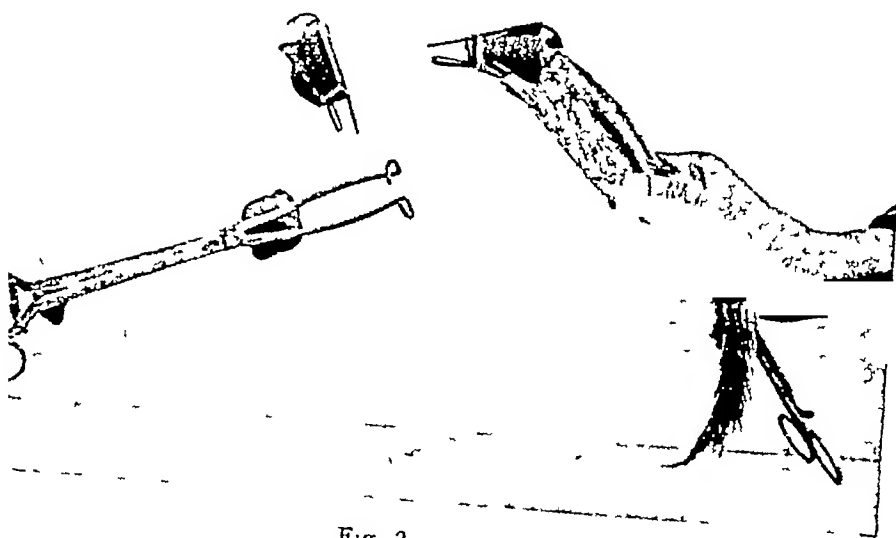


Fig 2

infection. If, however, this interpretation is not accepted then there is evidence that the virus is alive in an attenuated form after exposure to ether vapour for 120 hours. Longer periods were not tested. A comparison of the results obtained by immersion and exposure to vapour only tends to show, as might be expected, that immersion of the brain in the fluid has a more regular and certain action in the virus than has exposure to the vapour alone. It is possible, therefore, that signs of life might still have been present after exposure to the vapour for 144 hours or for even longer periods.

The experiments recorded here show that the virus of the street is more resistant to the action of ether than is the Kasauli fixed virus, and the results obtained with this type of virus are more in keeping with those recorded by observers in Europe.

#### SUMMARY

The resistance to ether of the virus of the street has been tested both by immersion of infected brains in the fluid and by exposure to the vapour with the following results —

1. Positive evidence of the viability of 'street virus' in the central portions of the brain was obtained in brains immersed up to 72 hours.

2. After this period the evidence for the presence of living virus depends upon the interpretation placed upon the deaths which occurred after infection with material immersed for longer periods. If these deaths are held to be due to an attenuated virus then the virus apparently remains alive up to 120 hours but is killed after a 144-hour immersion.

3. The action of ether vapour on 'street virus' is very similar to that recorded above for the immersion of infected material, but the action of the vapour appears to be less even and probably not quite so rapid in its results.

4. The strains of 'street virus' tested are more resistant to ether than the fixed virus in use in this Institute.

5. The results obtained in these experiments approach more nearly those recorded by European observers.

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# AN INVESTIGATION INTO THE VALUE OF AN ETHERISED VACCINE IN THE PROPHYLACTIC TREATMENT OF RABIES

## Part II.

### THE ACTION OF ETHER ON 'STREET VIRUS' IN INFECTED BRAINS

BY

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(*Investigation aided by a grant from the Indian Research Fund Association*)

[Received for publication, February 21, 1927]

IN our last paper we described the action of ether on the fixed virus in use in Kasauli and found that it was much less resistant than the virus in use at certain Pasteur Institutes in Europe

In the present note we propose to give the results of certain experiments designed to test its effect on brains infected with 'street virus' According to Alivisatos ether appears to have very much the same effect on 'street virus' as it has on fixed virus and the times required for its various effects are apparently very similar in each case

The 'street virus' we have used for these experiments has been obtained from two sources

Virus 1 In its 1st subpassage in the rabbit produced symptoms in 12 and death in 18 days The 3rd subpassage of this virus was used for the 1st experiment (Table I) and gave very similar periods for symptoms and death

Virus 2 Which was used for the 2nd experiment (Table II) produced symptoms on the 7th day and death in 12 days

piebalds were less susceptible than the jet black, but the jet black rabbits were not so susceptible as the Belgian hare breed

The results of this insulin assay on these rabbits can be summarized in the following table (For detail results see Appendix I, II, III and IV)

TABLE I

Breed	PERCENTAGE REDUCTION OF BLOOD SUGAR 2 HOURS AFTER INJECTION OF INSULIN (DOSE PER KILO)		
	Maximum	Minimum	Average
Albino Himalayan	55	30	48
Black and white piebalds	57	50	55
Jet black	70	51	60
Brown Belgian hare	82	57	66.7

This table shows that the albino Himalayan rabbit is the least susceptible and the Belgian hare rabbit the most. In fact the rabbits of the Belgian hare breed began to show hypo-glycæmic reactions at the end of the first hour after the injections of insulin, and many of them had to be revived by intravenous injections of glucose. The jet black rabbits came next as regards susceptibility, and 4 out of the 13 showed hypo-glycæmic reactions, and one of them died as a result of hypo-glycæmia in spite of the glucose. In none of the white rabbits was there the least sign of hypo-glycæmia, in fact they all behaved like normal animals throughout the experiment.

The question which naturally arose from these results was what is the cause of the variability? In the Himalayan breed it was obviously due to some association with the colour of the rabbit. It is a known fact that colouration of the animal is closely associated with the function of the medullary substance of the suprarenal glands. Thus the hair of the Arctic fox turns white during the winter and reverts to a dull brown during the summer, a condition also correlated with the stress of life that occurs during these seasons in the Arctic zone.

The literature contains a large amount of evidence to show that the action of adrenalin is directly antagonistic to that of insulin as it allows a larger amount of glucose to be available as blood sugar from the liver. It seems, therefore, highly reasonable to suggest that the high adrenalin content of these albino rabbits was capable of inhibiting the action of insulin to a large extent. We also found that, in human beings suffering from leucoderma, these people had a lower blood sugar content like the European and responded less to the injection of insulin. In conditions of hyper-pigmentation we found that these patients reacted rather powerfully to injections of insulin.

In a previous experiment conducted by one of us (J. P. B.) it was found that small doses of adrenalin were capable of producing hyper-glycæmia in rabbits and that when adequate doses of both adrenalin and insulin were given simultaneously to the same rabbit there was neither a rise nor a fall in the blood sugar.

*Showing the effect of exposing 'street vir*

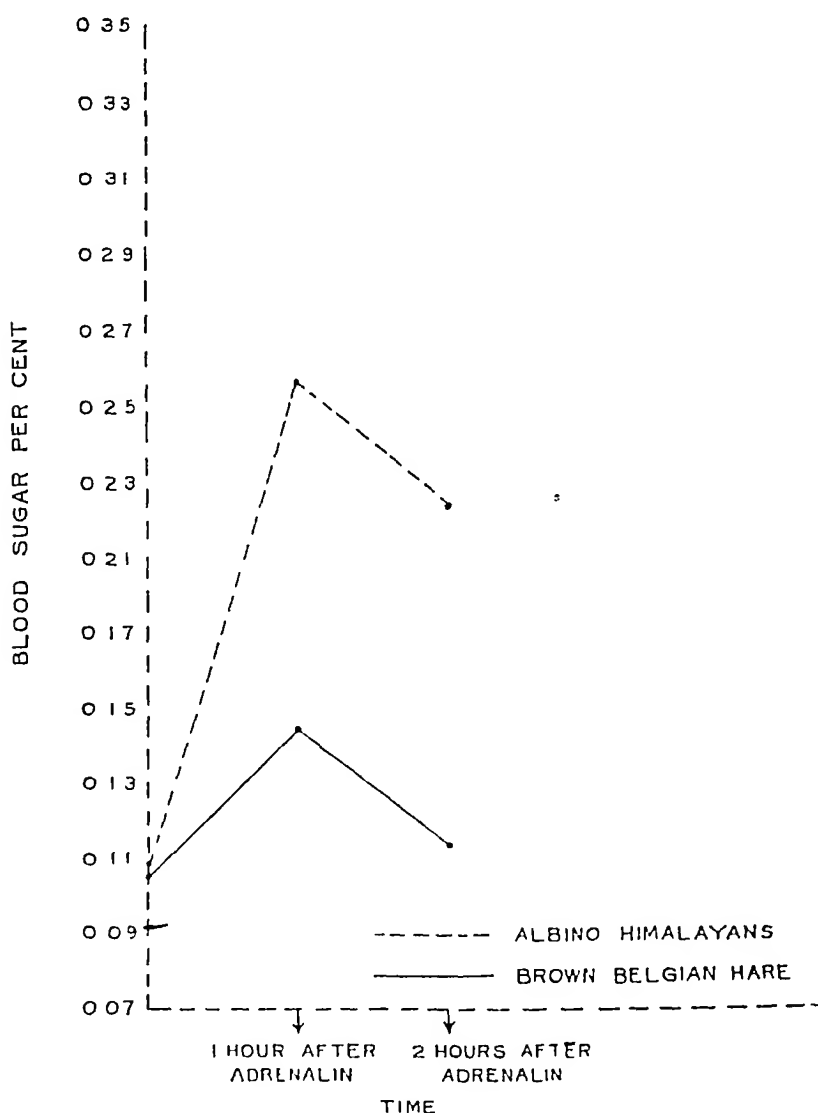
ETHERISED BRAINS			INOCULATION				
Number of hours exposed	Number of brains treated	Sample taken from	Number of rabbits inoculated	Death	Number of days to		
					First symptoms	Death	
24	1	Outside	1	1	9	10	St
		Inside	1	1	10	13	St
		Mixture	1	1	8	11	St
36	1	Outside	1	1	Paresis, hind legs	31	St
		Inside Mixture	1 1	1		2	St
48	1	Outside	1		Tremor?		
		Inside	1	1	14 11	12	Co
		Mixture	1	1	9	11	St
72	1	Outside	1	1	28	29	St
		Inside	1	1	7	8	St
		Mixture	1	1	Tremor	5	St
84	1	Outside	1	1	163	165	Co
		Inside	1	1	Suddenly	115	St
		Mixture	1	1	70 paresis, hind legs	84	St
96	1	Outside	1	1		29	St
		Inside Mixture	1 1				
120	1	Outside	1	1		17	St
		Inside	1	1	39 paresis, hind legs	81	St
		Mixture	1	1		1	St



It will be seen that in the albino Himalayans, the blood sugar rises from 0.11 to 0.256 after the first hour and falls to 0.224 in the second hour as is shown by the dotted line in the chart

In the brown Belgian hare rabbits on the other hand, the blood sugar rises after the injection of adrenalin from the initial level of 0.106 per cent to 0.145 per cent only, and falls very near to the normal level (0.114 per cent) in the second

GRAPH I



hour. This experiment clearly indicates that adrenalin acts very powerfully on the albino rabbit, liberating a large amount of glucose from the glycogen store-house of the liver which is capable of antagonising the action of insulin. In the brown Belgian hare rabbit, the adrenalin response by the liver towards adrenalin is very much less than in the albino, so that insulin is then capable of reducing the blood sugar from 0.11 to 0.03 or 0.04 and producing the symptoms of hypo-glycaemia.

The brains were either immersed in the ether fluid or exposed to the vapour for varying periods and then portions from the outside, inside and a mixture of the whole brain were inoculated into other rabbits subdurally.

Specimens of the brains of the rabbits which died were either searched for the presence of negri bodies or else were subpassaged into other rabbits.

The presence of negri bodies was taken as positive evidence of rabies and in such cases no subpassages were done.

The rabbits which escaped were all infected subdurally with fixed virus at varying periods after 3 months. (The exact dates are given in the tables.) None of these animals proved refractory to rabies infection.

The first table gives the results of the immersion of the brains in ether for varying periods of time.

Twenty-four hours' immersion has had little effect on the virus, for the specimens taken from all these positions were capable of conveying the disease by subpassage to other rabbits without any appreciable prolongation in the period of symptoms and death. After 24 hours, however, the virus in the outer portion of the brains appears to have been killed, for with the exception of the 84-hour specimen, none of the animals subpassaged with material from this position have died. A gradual attenuation from without inwards is evident in the specimens immersed up to 72 hours, both the inside and mixture being virulent in the 48-hour specimens and the inside portion alone in the 72-hour brain. After 72 hours' immersion it has been impossible to demonstrate the presence of living virus with certainty. Animals inoculated with material immersed for longer periods have certainly died but negri bodies have not been found and subpassages have either failed to die or have died without showing typical symptoms. Harvey and Acton consider that many of these deaths are due to the inoculation of foreign nerve tissue alone, and have nothing to do with the rabies virus. If this interpretation is accepted then there is no positive evidence of the virus remaining alive after immersion for 72 and possibly 84 hours. On the other hand if the irregular deaths which have occurred in the subpassages from the '96-hour' brain onwards are held to be due to a very attenuated virus, then there is evidence that the virus remains alive after immersion for 120 but not 144 hours. This latter supposition would bring our results more in line with those of the European observers.

The results obtained from exposure of the brains to ether vapour (Table II) are not so clear cut due probably to the fact that the vapour does not penetrate so evenly as the fluid. Attenuation and death of the virus, however, appears to proceed, generally speaking, at much the same rate as when the brains are actually immersed in the fluid. Thus the subpassages die with typical symptoms after inoculation with material exposed to the vapour up to 72 hours. After this period, although more animals have died than in the 1st experiment, the symptoms both in the 1st and 2nd subpassages have not been typical of the disease. These deaths are open to the interpretation already mentioned above that they are not due to rabies.

## APPENDIX II

*Effect of insulin on the blood sugar of piebald Himalayan rabbits*

(Dose = 3 units per kilo)

Serial number	Weight in grammes	Blood sugar two hours before (percentage)	Blood sugar two hours after (percentage)	Percentage of reduction
1	1,770	0.102	0.050	50.9
2	1,990	0.106	0.050	52.8
3	1,910	0.119	0.051	57.1
4	2,280	0.112	0.049	56.2

Minimum reduction	50 per cent
Maximum reduction	57 per cent
Average reduction	55 per cent

## APPENDIX III

*Effect of insulin on the blood sugar of jet black rabbits*

(Dose = 3 units per kilo)

Serial number	Weight in grammes	Blood sugar before (percentage)	Blood sugar two hours after (percentage)	Percentage of reduction	REMARKS
1	1,670				
2	1,780	0.146	0.044	70	Tremor, restlessness, rapid breathing Slight convulsions Tremor, listlessness Not moving when touched
3	2,220	0.129	0.051	61	
4	1,750	0.129	0.058	55.1	
5	1,880	0.129	0.051	61.0	
6	1,690	0.145	0.051	65.0	
7	1,550				
8	1,270	0.155	0.061	60.6	
9	1,220	0.136	0.044	68.0	Had severe hypo-glycæmic reaction, convulsion Glucose injection given but died
10	1,380	0.129	0.039	70.0	
11	1,780	0.119	0.050	58.0	
12	1,580	0.107	0.052	52.0	
13	1,080	0.100	0.049	51.0	
14	980	0.100	0.048	52.0	

Minimum reduction	51 per cent
Maximum reduction	70 per cent
Average reduction	60 per cent

# THE RELATIONSHIP OF THE COLOUR OF RABBITS TO THEIR SUSCEPTIBILITY TO INSULIN

BY

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IN 1925, whilst dealing with the question whether insulin deteriorated in the tropics, we noticed during the course of our experiments that several of our animals reacted very differently to the same dose per kilo weight. One of us (H W A) noticed during the course of preparing antivenom, using goats for immunization, that the white goats were more susceptible to cobra venom than the black goats. We likewise found that the Himalayan rabbits of different colours which were used by us for the assay of insulin behaved quite differently when the same dose of insulin was injected per kilo of body weight. We extended the experiments on a larger scale in order to eliminate the possibility of individual variation, and we also include the brown Belgian hare rabbit as a separate breed. The following types of rabbits were used for our experiments —

- (a) Himalayan
  - (1) albino,
  - (2) piebald,
  - (3) jet black rabbits, and
- (b) Belgian hare rabbit

The most noticeable fact was that the white rabbits were least affected by the injections of insulin, whilst the Belgian hare rabbits were extremely susceptible, and 10 out of the 13 got severe symptoms of hypo-glycæmia, and one of them (No 8) died of it, in spite of the fact that an intravenous injection of glucose was administered to it. In the Himalayan breed, we found that the



With a view to finding indirectly the adrenalin response of these rabbits of different breed and colouration we proceeded to see the effects of injections of adrenalin on the blood sugar. The same dose, i.e., 0.25 c.c., were given to all the rabbits and the blood sugar was examined both before and after the adrenalin injection.

Graph I shows the results of the effect of adrenalin injections on the blood sugar of the albino rabbits and the sensitive brown Belgian hares. The results are shown in detail in Tables II and III.

TABLE II  
*Albino Himalayans*

Serial number	PERCENTAGE OF BLOOD SUGAR		
	Before	One hour after adrenalin	Two hours after adrenalin
White 1	0.112	0.280	0.228
White 2	0.125	0.256	
White 3	0.083	0.201	0.263
White 4	0.088	0.224	0.141
White 5	0.116	0.211	0.160
White 6	0.096	0.280	0.220
White 7	0.112	0.240	0.154
White 8	0.128	0.296	0.234
White 9	0.133	0.312	0.328
White 10	0.106	0.264	0.288

*Average of Results*

Blood sugar before	0.109 per cent
Blood sugar one hour after adrenalin	0.256 per cent
Blood sugar two hours after adrenalin	0.224 per cent.

TABLE III  
*Brown Belgian hare*

Serial number	PERCENTAGE OF BLOOD SUGAR		
	Before	One hour after adrenalin	Two hours after adrenalin
Brown 1	0.085	0.095	
Brown 2	0.074	0.105	0.068
Brown 3	0.070	0.116	0.096
Brown 4	0.137	0.198	0.112
Brown 5	0.101	0.151	0.145
Brown 6	0.132	0.212	0.168
Brown 7	0.148	0.160	0.124
Brown 8	0.128	0.176	
Brown 9	0.080	0.096	0.085

*Average of Results*

Blood sugar before	0.106 per cent
Blood sugar one hour after adrenalin	0.145 per cent
Blood sugar two hours after adrenalin	0.114 per cent.

The first definite case of the nodular stage of this condition was described by Brahmachari (1922) who suggested the name 'dermal leishmanoid,' a second case was described by Knowles, Napier and Das Gupta (1923), and a third by Shortt and Brahmachari (1925)

The 'xanthoma' type of this condition was first observed by the senior writer and a short note accompanied by two illustrations of the first case appeared in the Annual Report of the Calcutta School of Tropical Medicine for 1925

Since the autumn of 1925 a number of cases of all stages of the disease have been recognised in the out-patient departments of the School. A certain number of these patients had attended previously and some other diagnosis had been made but the majority attended for the first time during this period

It is a matter of some interest to speculate why 44 cases of a disease of which we have only seen about half a dozen cases previously should come to light in a period of about a year. The condition is so characteristic in all its stages that a clinical diagnosis can be made in almost every instance

The cases described in this paper do not include the few cases seen by us before November 1925, as full details of these cases are not available

### ÆTIOLOGY

The disease is almost certainly a sequel to a generalised infection with the parasite *Leishmania donovani*. More than half the patients give a definite history of having had kala-azar and having received treatment for this disease, other patients give a history of having had fever of some months' duration accompanied by enlargement of the spleen some years previously and in a few cases there is no history of either kala-azar or long-continued fever. It is known that a certain number of patients suffering from kala-azar recover without the administration of any specific treatment and that transitory leishmaniasis manifested by only a short bout of fever does occur. The fact that in a number of cases there is no definite history of kala-azar does not suggest to the writers that these skin lesions may occur independently of a generalised leishmaniasis, but that instances of transient leishmaniasis and of spontaneous cure in clinical kala-azar are more frequent than was previously supposed

All the patients have been living in a kala-azar endemic area almost continuously

In every instance in which the examination has been made a parasite indistinguishable from *L. donovani* has been found in smears from the nodules and in many a culture of leishmania has been obtained from the lesions

Not only is the parasite morphologically indistinguishable from *L. donovani* but in culture it behaves like this organism and a generalised infection has been caused in a mouse by intraperitoneal injection of a culture of this parasite (Das Gupta, 1927). It has not yet been shown whether the organism has undergone any serological change during its existence in skin lesions in man

There is nothing in the race, class, sex or age distribution of the patients to suggest that there is any other factor which predisposes the kala-azar patient to the subsequent development of this condition. Females predominate relatively amongst patients suffering from the early lesions, this is natural as the slight

## CONCLUSIONS

(1) In rabbits of the same species but of different colour variations, insulin causes a smaller reduction in the blood sugar in the albino than in the black rabbits

(2) The reduction of blood sugar in the black Himalayan rabbits is so lowered as to produce hypo-glycæmia. This difference in the colour variations of the same species is due to the fact that adrenalin content in the blood of the albino animal is higher than in the black

(3) As regards the different species, the Himalayan rabbit is less susceptible to insulin than the brown Belgian hare rabbit, in fact the latter behaves towards insulin like the melanotic variety of Himalayan rabbit

(4) The brown Belgian hare rabbit responds poorly to the injections of adrenalin, so that the blood sugar is only slightly raised above normal

(5) There is some association between the colour and species as regards the output of adrenalin from the adrenalin glands. In albino animals the output is high, in the melanotic animals the output is low

## APPENDIX I

*Effect of insulin on the blood sugar of albino Himalayan rabbits*  
(Dose = 3 units per kilo)

Serial number	Weight in grammes	Blood sugar before (percentage)	Blood sugar two hours after (percentage)	Percentage of reduction
1	1,390	0.102	0.068	34.0
2	1,550	0.102	0.068	34.0
3	1,800	0.112	0.068	39.3
4	1,450			
5	1,100	0.086	0.060	30.2
6	1,370	0.072	0.052	27.7
7	1,160	0.100	0.052	48
8	1,200	0.095	0.056	41.05
9	1,450	0.085	0.054	36.4
10	1,320			
11	1,590	0.107	0.053	50.6
12	1,370	0.123	0.062	49.6
13	1,530	0.100	0.048	52.0
14	1,440	0.121	0.054	55.37
15	1,330	0.112	0.076	32.1

Minimum reduction

Maximum reduction

Average reduction

30 per cent

55 per cent

48 per cent





## APPENDIX IV

*Effect of insulin of the blood sugar of Belgian hare rabbits*

(Dose = 3 units per kilo)

Serial number	Weight in grammes	Blood sugar before (percentage)	Blood sugar two hours after (percentage)	Percentage of reduction	REMARKS
1	1,010				
2	1 210	0 146	0 044	70 0	Severe reactions Convulsions, relieved with intravenous glucose
3	1 550	0 100	0 018	82 0	Severe convulsions Animal lying with head retracted Intravenous solution of glucose (20 c c of 5 per cent solution)
4	1,600	0 121	0 040	67 0	Severe convulsions at intervals of 2 minutes lasting for about one minute each time Saved with intravenous glucose.
5	1 510	0 100	0 043	57 0	
6	1,530	0 112	0 032	80 3	Severe convulsions Retraction of the head and extension of the hind limbs Intravenous glucose given
7	1,470	0 100	0 033	67 0	Severe convulsions Intravenous glucose given
8	1,810	0 112	0 032	80 3	Severe convulsions, hurried respiration H R over 156, died in spite of intravenous glucose
9	1 630	0 116	0 048	58 6	Slight convulsions
10	1,440	0 137	0 060	57 0	Very severe convulsions H R over 160 Rabbit gasping for breath Intravenous glucose given
11	1,460	0 121	0 050	58 6	Severe convulsions Intravenous glucose given
12	1 690	0 132	0 048	63 6	
13	1,840	0 120	0 048	60 0	

Minimum reduction

57 per cent

Maximum reduction

82 per cent

Average reduction

66.7 per cent



# POST-KALA-AZAR DERMAL LEISHMANIASIS

BY

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## HISTORY

It seems possible that the cases reported by Thomson and Balfour (1909) were really cases of this condition. These writers refer to the lesions as 'soft, pink, keloid-like raised growths' showing no tendency towards ulceration, this is a description which could be applied to many of the nodular lesions in the cases described below. Parasites, which the writers assumed to be *Leishmania tropica* but which might equally well have been *Leishmania donovani*, were found in the lesions in large numbers. The patients were soldiers in the Egyptian army, they came from upper Egypt and had been serving in the Sudan for some months. They came from an area where *L. tropica* infection is unknown, or very rare. Kala-azar occurs sporadically in the Sudan but is not reported from upper Egypt.

Carter (1911) and others have described non-ulcerating leishmania-infected lesions of the skin in patients living in various districts in India where oriental sore is endemic. The history of the onset of these lesions is the typical history of the first appearance of an oriental sore, a small red pimple appears, painless but itching, and later this is surrounded by a red zone. The lesions themselves when well developed appear to be quite different from the lesions of the condition which we are describing and in view of their association with oriental sore it is obvious that the condition is a slight variation from the normal clinical manifestation of *Leishmania tropica* infection.





Fig 1 The arm of a girl showing minute depigmented dots the earliest stage of this condition

than in other parts of the body. The sites of appearance of the nodules in the order of frequency are as follows—Chin, cheeks, nose, lips, neck, forehead, extensor surface of forearms, upper arm, ears, side of chest, back, legs, thighs, hands and feet. The depigmentation is usually very marked on the inner side of the thigh but here the condition seldom goes on to nodular formation. The nodules vary in size but are usually the size of a split pea. The very extensive nodules that sometimes occur are probably due to the coalescence of a number of smaller nodules. The nodules are soft yellowish-pink granulomatous growths, they show no signs of ulceration and are painless. The lesions occasionally extend on to the mucous membranes, especially those of the lips and nose (Plate XVI, fig 11). In one instance the mucous membrane of the pharynx appeared to be involved.

In this stage the disease is frequently mistaken for leprosy which in some instances it simulates very closely.

The condition is very chronic and the lesions undergo very little change over long periods (Plate XVII, figs 12 and 13).

*The xanthoma-like stage* (Plates XX and XIX, figs 14 and 15)—From the comparative rarity of this condition, it is obvious that it is not the inevitable sequel to the nodular stage but in all the cases showing this condition the history is that the patient suffered from nodules for some years before the xanthoma-like condition appeared. The patients showing this condition usually give a history of lesions lasting for 10 years or more.

Both the distribution and nature of the lesions are very similar to those of the condition known as xanthoma tuberosum multiplex. The sites where the most characteristic lesions are usually seen are the axillary folds, the ante-cubital space, the inner side of the thigh, the outer canthus of the eye, the eyelids and the chin, but they may occur on almost all parts of the body. The lesions are slightly raised orange coloured plaques, often quite small but sometimes as large as the palm of one's hand, they are painless and there is no ulceration.

#### DIFFERENTIAL DIAGNOSIS

The distribution and colour of the lesions are characteristic and makes the differential diagnosis easy provided one is familiar with the disease. The diagnosis is best considered according to the stage of the skin lesions.

I *Depigmented stage*—In this stage when leucodermic spots only are present one has to differentiate from simple leucoderma, syphilitic leucoderma and white spot disease.

*Simple leucoderma*—This disease commences with a herald spot which is soon followed by a similar spot on the opposite side of the body. The depigmentation is complete. The areas are large and usually symmetrical in distribution. There are four types of leucoderma. The melung type—affecting the hands and feet, the muco-cutaneous type—affecting muco-cutaneous junctions, the dhoti type—from pressure of the loin cloth and, finally, a generalised type. In dermal leishmaniasis the leucodermic areas are not completely depigmented and commence as small spots in the chin, cheeks, front of the chest, arms and inner sides of the thigh.









Fig 5 Small boy showing wide-spread depigmented lesions with raised areas here and there the pre-nodular stage.



disfiguring effect of these lesions would not be as serious a matter to a man as to a woman

The first two cases of this form of dermal leishmaniasis that were recognised both happened to have a positive Wassermann reaction and it was suggested that syphilis might predispose the patient to that condition, subsequent experience has added no support to this suggestion

It was also suggested that the condition might be caused by the treatment by sodium antimony tartrate (Brahmachari, 1922) but, although many of the patients in this series had sodium antimony tartrate, a few only had urea-stibamine injections and many no antimony treatment whatsoever

Although everything points to the lesions of this condition having been caused by *L. donovani* it is not clear how and why this organism establishes itself in the skin. The natural assumption is that the parasite gained entry into the body prior to the patient's attack of clinical kala-azar, that when the patient's resistance overcame the general infection the parasite took refuge in certain cells in the skin, and that here they remained eventually multiplying and causing the lesions described below

There is of course the possibility that a second invasion of leishmania occurred but that the patient's natural resistance having been raised by a previous attack of kala-azar the parasites were unable to cause a general infection and were only able to obtain a footing in the skin, or in other words that the skin lesions are the clinical manifestation of a second invasion of *L. donovani*. In favour of this suggestion is the fact that all the patients have continued to live in the endemic area, but against it is the fact that a definite period of about one year always elapses between the appearance of the symptoms of kala-azar and the first appearance of the skin lesions

#### DESCRIPTION OF THE LESIONS

There are three distinct types of skin lesion which represent three stages in the development of the disease, namely —

*The first, or depigmented, stage* (Plates XI, XX and XII, figs 1, 2, 3 and 4) — The depigmented patches usually appear on the face, most commonly on the chin and lips, on the neck, on the extensor surfaces of the forearms, on the inner sides of the thighs and eventually on all parts of the body. They appear first as minute dots enlarging until they become in some cases irregular areas about half an inch in diameter. In those cases in which there is a history of kala-azar, the lesions are usually first observed about one year after the patient has completed the course of treatment for kala-azar. The depigmentation is not complete, as in the case of leucoderma, but in the Indian and Anglo-Indian the affected areas are distinctly lighter than the normal skin, we have not yet seen the condition in a European. After a few months the depigmented areas become slightly raised and eventually pass into the next stage, the nodular stage

*The second, or nodular, stage* (Plates XIII, XIV, XV and XVI, figs 5, 6, 7, 8, 9 and 10) — The usual history is that the nodules appear about two years after the kala-azar treatment is completed. Although the nodules replace the depigmented patches, there are certain areas where the nodules appear much earlier





Fig 6—Extensive nodular lesions on face, this condition resisted all treatment of treatment



Fig 7—Small girl with numerous nodules on the face, Case No 34

In this stage no parasites can be seen in the sections, nor can they be seen in smears made from deep scrapings, but on two occasions cultures of leishmania were obtained by dropping a small portion of depigmented tissue into NNN medium

*The nodular stage*—Sections of the nodules show that the epithelium is thin and the papillæ flattened, and that it consists of a basal layer containing very little pigment, a couple of layers of prickly cells and a thin covering of horny cells. The sub-papillary layer is cedematous and the fibrous and elastic tissue has atrophied, melanoblasts are well seen. Below this cedematous area is a granulomatous mass consisting largely of proliferating macrophages and fibroblasts. Here and there in the centre of the mass there are multi-nucleated cells packed with parasites, away from the centre of the nodule the macrophages contain fewer parasites and the cells at the periphery do not appear to be parasitised.

It is very easy to demonstrate the parasites in this stage. A smear made from a deep scraping will usually reveal large numbers of parasites. The best method of demonstrating the parasites is, however, by cutting of a small portion of a nodule with a pair of sharp curved scissors, the under surface of the portion of nodule is smeared on to a slide and the smear stained either by Leishman's or Giemsa's stain (Plate XXII, fig. 17). In some cases it will be necessary to search for the parasites but in the majority they will be seen almost immediately lying in the macrophages. A few free forms will usually be seen but these have almost certainly come from ruptured macrophages.

*The xanthoma like stage*—In the nodular type the spread is centripetal, raising and flattening the epidermis, in the xanthoma stage the tendency is for the spread to be centrifugal with the formation of large plaques in these areas. The histological appearances are similar to the nodular type but there is a tendency to fibrosis and constriction of the venules with subsequent dilatation, for this reason the xanthoma plaques are a deeper orange red colour while the nodules are a paler orange yellow colour.

The parasites can be demonstrated in the same way as in the nodular stage, they are usually numerous.

*Parasites in the peripheral blood*—Parasites have never been observed in the peripheral blood by direct examination of the films but in two cases a growth of leishmania has been obtained on NNN medium. This is by no means a constant finding as it is in kala-azar and the presence of the parasites in the peripheral blood is probably more or less an accident but it may mean that the patient suffering from this condition can act as a carrier.

*The blood picture*—The leucopænia which is characteristic of kala-azar is not present in this condition, in fact in a number of cases there is a distinct leucocytosis. The only other characteristic feature of the blood is an eosinophilia, the eosinophil count is usually between 500 and 1,000 per cmm and in some instances is higher than this.

*The aldehyde reaction*—There is usually solidification of the serum within 24 hours and in some instances there is a slight haziness but in no instance has anything like a positive reaction been observed.

*The Wassermann reaction*—This test was not carried out as a routine measure but in view of the fact that the first two cases of this condition reported

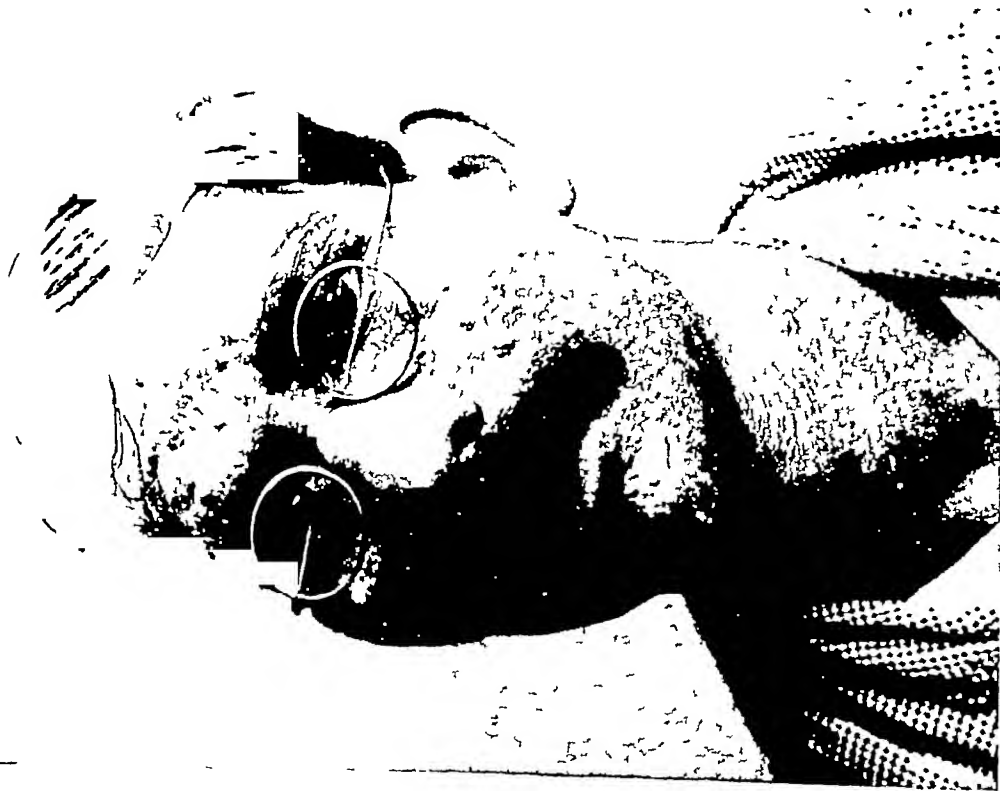


Fig 8—Nodular lesions on the face, Case No 20

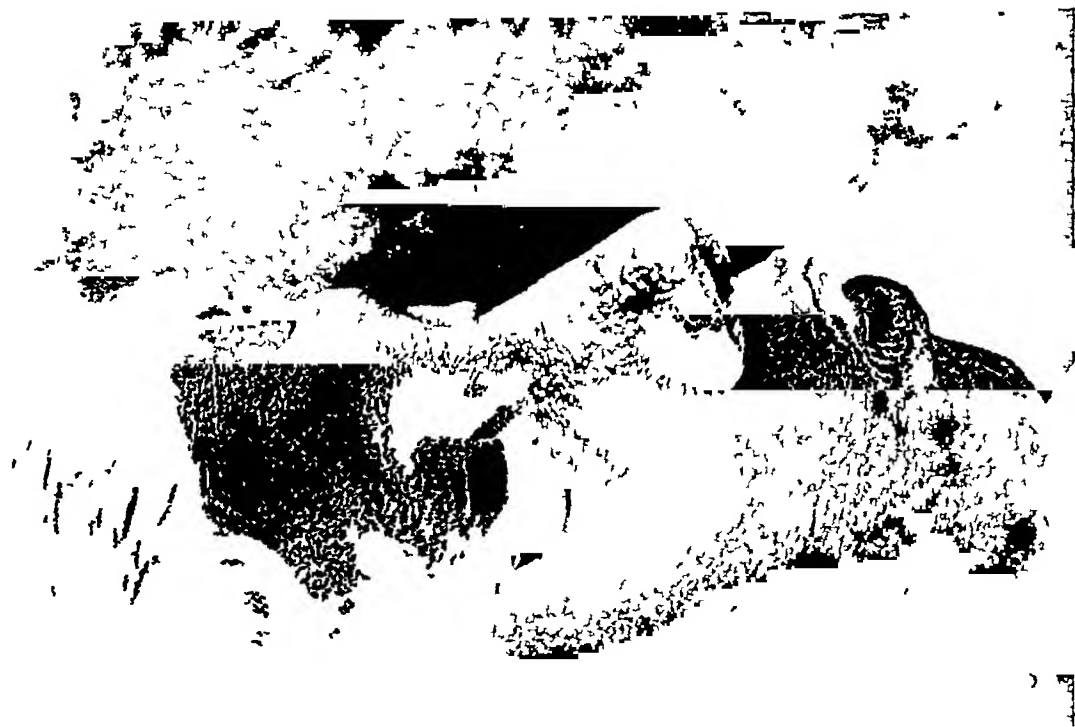


Fig 9—A boy showing nodules on the nose, lips and chin and depigmented areas on the cheek and chin







Fig 10—Case showing the formation of large nodules on the nose and cheek, Case No 22



Fig 11—A man showing nodules on the face There is one large nodule inside the nose, Case No 44



• *Syphilitic leucoderma*—This is really a chloasma distributed on the forehead, back of the neck, etc., where the areas of hyperpigmentation show up the lighter skin, the condition is not noticeable on dark skins

*White spot disease*—This is a guttate type of morphea and is seen as small atrophic scars situated on the side of the face, neck and front of chest

It is seldom necessary to resort to the cultivation of the parasite from the leucodermic areas in order to confirm a diagnosis of dermal leishmaniasis

II *The nodular stage*—The disease which this condition most closely simulates is nodular leprosy of the face, however, the orange-yellow colour of the lesions, the absence of anæsthesia, the absence of thickening of the great auricular nerve and the co-existence of depigmented areas, characteristically distributed, in dermal leishmaniasis readily distinguish this condition from leprosy

Syphilis need hardly be considered, as small gummata of the skin soon ulcerate, whilst ulceration of the lesions of dermal leishmaniasis never occurs

We have seen one case of adenoma sebaceum of Pringle's type simulate this condition on superficial examination, there were raised nodules on the side of the nose, upper lips and naso-labial sulci with redness of the neighbouring skin

One has seen so many mistakes made in the diagnosis of skin diseases that it is well to repeat the dictum that most of the errors made are not due to ignorance but to insufficient examination. The combination of nodules on the face with leucodermic areas on the arms and thighs should at once suggest something unusual and call for further examination by smears and sections

III *Xanthoma type*—The lesions are typical of the text-book description of xanthoma tuberosum multiplex, in fact the first case seen by us was diagnosed as such, section of the lesions in this case showed the xanthomatous cells with the so-called xanthomatous bodies therein. Whilst this patient was in hospital a second case came to Dr Muir, diagnosed as leprosy. As there were no acid-fast bacilli but only peculiar nuclear bodies, the case was referred to the senior author when fresh smears were made, fixed with methyl alcohol and stained by Leishman's stain, these nucleated bodies were seen to be leishmania parasite. The case in hospital was re-examined and leishmania parasites were also found in his lesions

The long history of the disease, 10 to 14 years, the deep orange colour of the lesions, the plaques on the axillary folds, bend of the elbow, and lesions on the mucous membrane of the throat make one wonder whether they are one and the same disease

#### PATHOLOGY

*The depigmented stage*—Histological sections show that the epithelium has undergone very little change but that there is less pigment in the cells of the basal layer in the depigmented areas than in those of the adjoining normal skin. There is oedema of the sub-papillary tissues and the vessels in this layer are large and dilated. Below this, there is a certain amount of infiltration by macrophages in the region of the sub-papillary plexus (Plate XXII, fig 16), the white elastic fibres have been destroyed in this area and it is probable that the function of the melanoblasts has been interfered with owing to the interference with their blood supply





Fig 12—Case No 28, in July, 1925



Fig 13—The same case as above, taken 18 months after the previous one

TABLE I—contd

OF THE LESIONS									Presence of Leishmania	HISTORY		
LIMBS				TRUNK				Duration of Lesions		History of Kala-azar or not	REMARKS	
Hands	Thigh	Legs	Feet	Neck	Back	Front of Chest	Abdomen					
0	0	0	0		0	0	0	0	+	6 months	Yes	Kala-azar about 18 months ago, treated by sodium antimony tartrate
0	0	0	0		0	0	0	0	+	1 year	No	History of fever some years ago
0		0	+		0	0	—	—	+	1 year	Yes	Kala azar 3 years ago, treated by sodium antimony tartrate 55 injections
0		0			0				+	2 years	No	Long continued fever some years ago.
		0							+		Yes	
0	+	0	0	+	0	0	0	0	+	10 years	No	Fever and splenic enlargement 10 years ago Xanthoma-like type of lesions at outer Canthus of eye
		0	0	0	0	0	0		+	2 years	No	Typhoid fever about 2½ years ago
	+					×	—		+	15 years	No	Severe attack of malaria 16 years ago
0		0	0			0			+	2 years	Yes	Kala-azar 3 years ago, treated 7 injections of urea-stibamine
0		0	0							2 years	No	Fever
0		0	0		+	0	0	0	+	4 years	Yes	Kala-azar 5 years ago
0	0	0	0	—	+	0	0	0	+			
0		0	0	—	—	+	0	—	—	1½ years	Yes	Kala-azar 2½ years ago, treated by 12 injections of urea-stibamine
									—		No	
0	0	0			0	0	0		+	3 years	Yes	Kala azar 4 years ago, treated at Shillong 52 injections of tartrate
					0				—	1 year	Yes	Kala azar 4 years ago, 40 injections sodium antimony tartrate
0	0	0	0		0	0	0	—	+	6 months	No	
0		0	0		0	0	0	—	—	9 months	Yes	Kala azar 3 years ago
0									+	4 months	No	Blood culture—Leishmania Cytitis—disappearing after antimony treatment
0		0	0		0	0	0	—	+	4 months	Yes	In hospital with kala azar 2 years ago, probably sodium antimony tartrate
—						0			+	2 months	No	Fever in 1919-1920
0	0	0	0		0	0			+	4 years	No	Fever 12 years ago

pigment patches  
 nodules  
 xanthoma-like lesions

— indicates that the particular lesions are not well marked

PLATE XVIII



Fig 18—Case No 5 before treatment

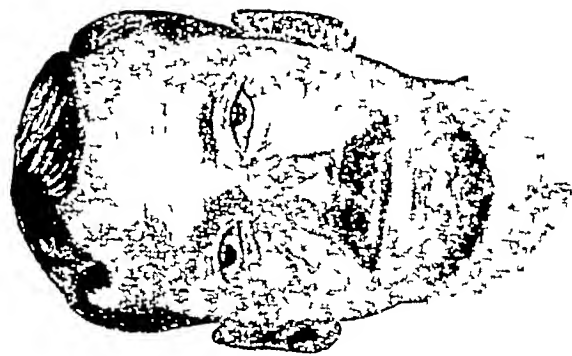


Fig 19—Case No 5 after 10 injections of Stibosan



last three years, two had been given a course of antimony treatment and had recovered, and one had died. He had had a few depigmented patches on his face and had taken no notice of them but during the last year nodules had begun to appear.

*Case No 35*—A Hindu doctor, aged about 47. Two and a half years ago he had kala azar and was given 12 injections of urea-stibamine which apparently cured him, his spleen became normal he had no return of fever and put on weight. About 18 months ago, one year after the attack of kala-azar, he noticed the depigmented areas on his face. He tried some of the various remedies that are advocated for leucoderma without obtaining any improvement. The nodules developed about 6 months ago. When seen by the junior writer the lesions were typically those of dermal leishmaniasis, smears from the nodules were not examined. The patient was advised to take antimony injections, he made his own arrangements for treatment.

*Case No 28* (Plate XVII, figs 12 and 13)—A Hindu male, aged 33, twelve years ago he had fever and enlargement of the spleen but did not receive any specific treatment for kala-azar. He had had nodules on his face for about 10 years. The large granulomatous mass at the root of his nose had commenced to grow about 8 years ago but had increased very little during the last few years. He attended the Out-Patient Department of the Calcutta School of Tropical Medicine 18 months ago and a provisional diagnosis of leprosy was made, a photograph was taken at the time. The diagnosis was not confirmed and the patient went back to his home in Sylhet. He returned to this institution and on this occasion the nature of the condition was recognised. Two photographs taken at intervals of 18 months demonstrate the chronicity of the condition, no specific treatment had been administered in the interval. After three weeks' treatment with urea-stibamine a very marked improvement in the condition was noticeable, the skin over the mass was loose and could be lifted up between the finger and thumb, whereas previously it had been tense and shiny. He then returned to his home.

#### ANALYSIS OF THE TABLE

*Race and religion*—Twenty-six of the patients were Hindus, twelve Mohammedans, four Anglo-Indians and two Jews. In a series of kala-azar patients attending the same institution analysed by the junior writer (Napier, 1922) the proportion was almost exactly the same except that in the earlier series there was a small percentage of Indian Christians, these seem to be replaced by Mohammedans in the present series. The percentages in the kala-azar series were, Hindus—58 per cent, Mohammedans—21 per cent, Europeans, including Anglo-Indians—13.7 per cent.

*Sex*—There were thirty-three males and eleven females, in the kala-azar series the percentage of women was only 15.

*Age*—In the case of five patients the age was not noted. Table II gives the numbers in each age group, the figures have been raised to percentages to facilitate comparison with the kala-azar series.

TABLE II

Age group	THIS SERIES		KALA-AZAR SERIES
	Number	Percentage	Percentage
Under 10 years	3	7.7	12
10 but under 20	14	35.9	40
20 but under 30	12	30.8	28.7
30 or over	10	25.7	19.3



Fig 15 Case No 1, showing extensive xanthoma-like lesions



Serial Number	Race and Religion	Sex	Age	Type of Lesions			DISTRIBUTION									
							HEAD AND FACE						Arms	Forearms		
				Depigmented Patches	Nodules	Xanthoma-like Plaques	Lips	Chin	Cheeks	Nose	Ears	Brow				
23	Moh	F	14	0	+		+	+	+				0			
24	Moh	M	11	0	+		+	+	+	+		+	0			
25	Hindu	M		0	+		0	+	+	0		0	0			
26	Moh	M		0	+		0	+	0	+		0	0			
27	Hindu	M	6	0	+		0	0	+	+		0	0			
28	Hindu	M	33	0	+	(x)	-	+	+	+		0	0			
29	Hindu	M	40	0	+		+	+	+		+		0			
30	Hindu	M	26	(0)	+	x	+	+	+			+	+			
31	Hindu	M	18	0	+			+	+	+		0	0			
32	Moh	M	15	0	(+)			0	(+)	0			0			
33	Hindu	M	18	0	+		+	+	+	+		+	0			
34	Hindu	F	8	0	+		+	+	+	+	+	+	0			
35	Hindu	M	47	0	+		+	+	+	+	-	+	0			
36	Moh	M	18	0	+			+	+				0			
37	Hindu	M	28	0	(+)			(+)	0	0	0	0	0			
38	Hindu	M	22	0	(+)			(+)	0	0		0				
39	Moh	M		0	+		0	0	+	+	+		0			
40	Moh	F		0	(+)			0	(+)	0	0		0			
41	Hindu	F		0	+		+	+	+		+					
42	Moh	F	17	0	+		+	+	+	+	-	+	0			
43	Hindu	M	27	0	+		+	+	-	+	+	0	0			
44	Moh	M	22	0	+		+	+	0	+	-	-	0			

0 =  
+ =  
x =

When these signs are between



in India had 'positive' Wassermann reactions the serum of 12 patients of this series was tested, in only one instance was the reaction 'positive'

### TREATMENT

It is not proposed to go into the details of the treatment of this condition in the present paper as our experience is limited and even in the cases in which a full course of treatment has been given it is too early to be dogmatic about the final results of treatment

Antimony appears to be the only substance that has any beneficial effect on the condition. Most of the compounds used in the treatment of kala-azar have been tried, in most cases with success. The patients that have not previously received treatment for kala-azar appear to react more readily. There is often marked improvement in the nodules after the first few injections have been given but the depigmented patches seem to persist for a longer time, they, however continue to progress towards recovery after treatment is discontinued (Plates XVIII and XXI, figs 18, 19, 20 and 21)

One patient, a nodular case previously treated for kala-azar, has proved entirely resistant to treatment, he has received between 50 and 60 antimony injections, antimony ionization, X-ray exposures and various forms of treatment by the mouth but there has never been any appreciable improvement in his condition

### THE CASES

The details of the 44 cases of the series have been arranged in tabular form but the histories of a few of these cases will be given in more detail

*Case No 1* (Plate XIX, fig 15)—A Hindu male, aged 27. The patient gave no history of having had kala-azar. Thirteen years ago nodules appeared on his face and afterwards on other parts of his body. He did not remember that these were preceded by depigmented patches. The nodules increased in size, coalesced with adjoining nodules and eventually became the characteristic orange-coloured plaques. The condition had undergone little change during the last few years. He was given two courses of 12 injections each of stibosan. After the first course of injections the condition had improved very markedly, the plaques were now flat although they remained discoloured and the isolated nodules had disappeared. Parasites were still found in some of the lesions so a second course was given. After the second course the discolouration of the skin only remained and no parasites could be found.

*Case No 12*—An Indian lady, a Hindu, about 30 years of age. She had lived in Bengal for a number of years, she gave no history of ever having had kala-azar but about five years ago she had fever lasting a month or so, which was treated as malaria. About three years ago she noticed a number of depigmented spots on her neck, face and forearms, later small nodules developed on her chin, nose and cheeks. She consulted a number of well-known specialists in Calcutta, one diagnosed the condition as leprosy and gave her 20 injections of hydnocarpus esters for this condition, another diagnosed syphilis and gave her two courses of six injections each of neosalvarsan, and yet others prescribed local applications. Her finances being exhausted she came to the Skin Out-patient Department of the School of Tropical Medicine where a diagnosis of dermal leishmaniasis was made, this was subsequently confirmed by finding the parasites in a smear from one of the nodules and the patient was given a course of 20 injections of stibosan. The nodules all disappeared and the depigmented areas were slowly assuming the colour of the normal skin at the end of the course.

*Case No 20*—An Anglo-Indian male, about 35 years of age. He gave no history of ever having had kala-azar but three of his children had suffered from the disease during the





Fig 14—Case No 30, showing nodular formation on the face and xanthoma-like plaques at the anterior axillary fold

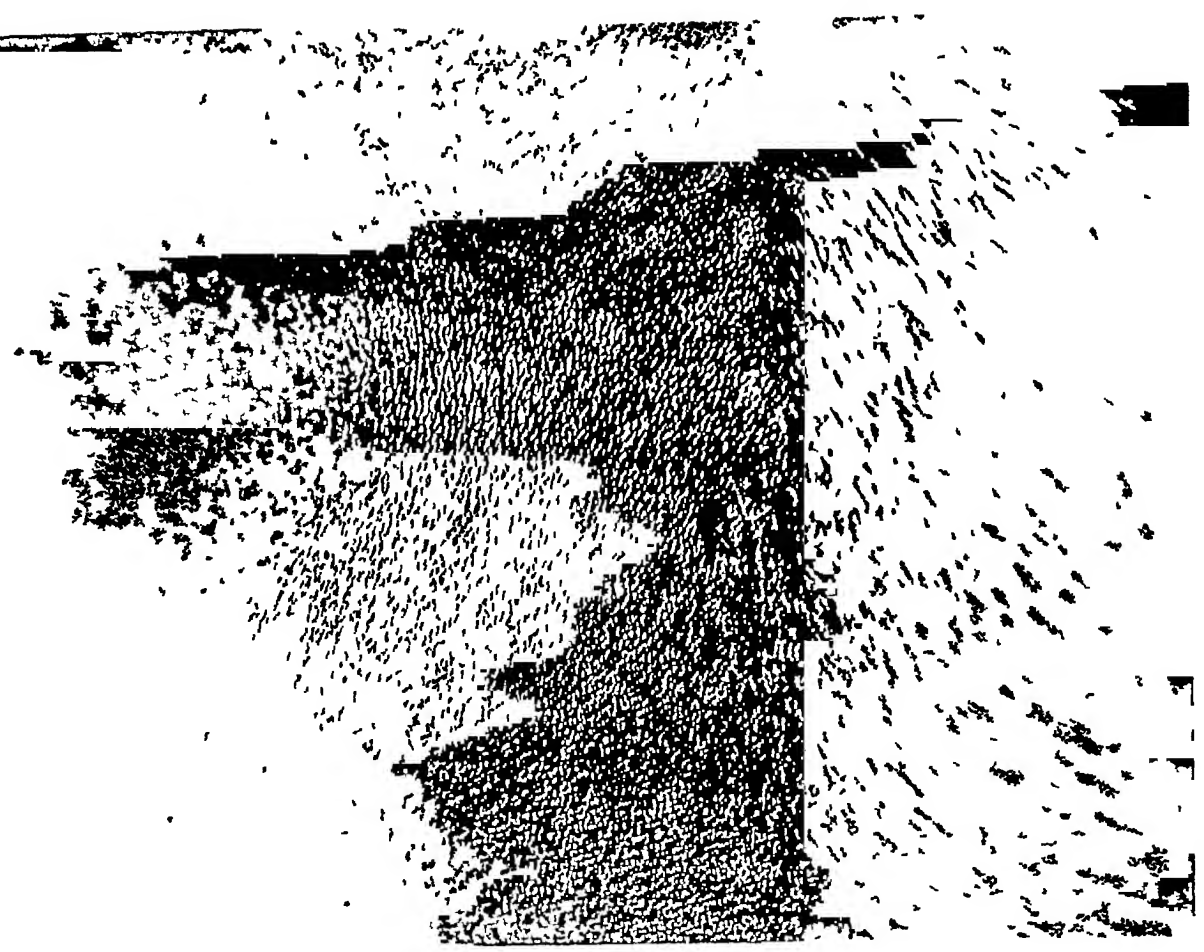


Fig 2—Well-marked depigmented patches on the back of the neck



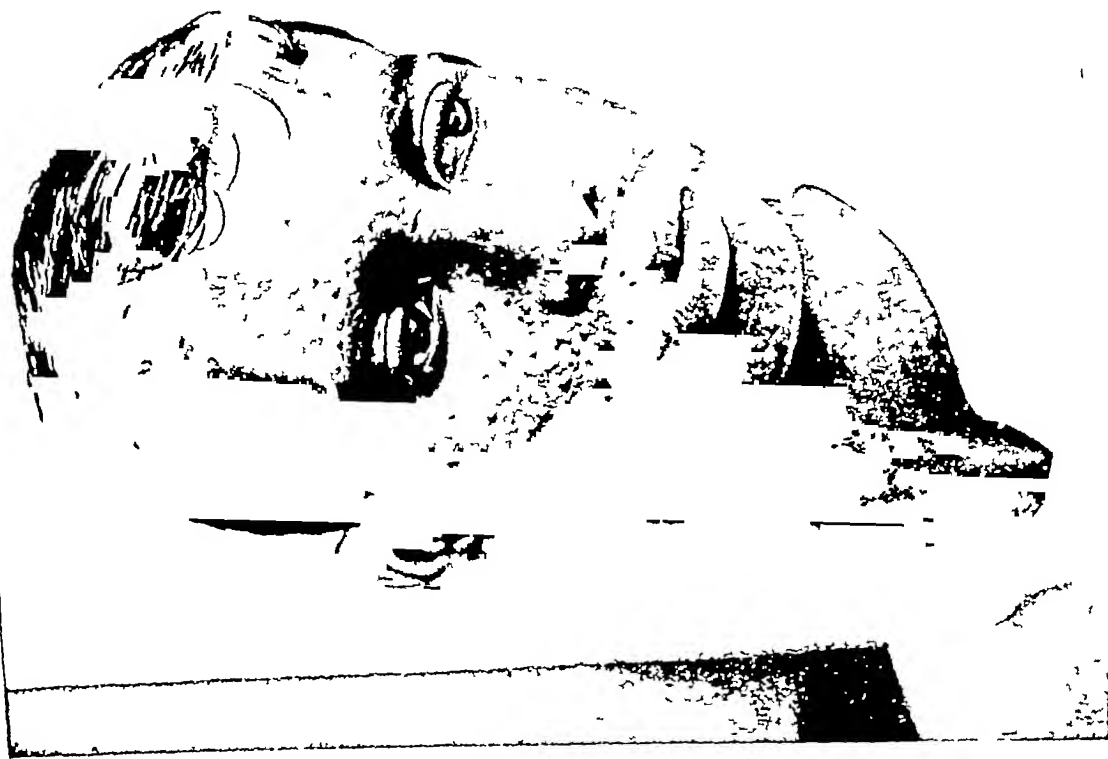
*The presence of parasites*—In 30 of the cases leishmania was demonstrated in the lesions. Nodular cases in which the parasites were not found on examination were not included in this series as the parasites are usually found in this type of lesion without difficulty, but in cases in which there are no nodular lesions the difficulty of finding the parasites in the depigmented lesions made it necessary to accept a clinical diagnosis, if the clinical appearances and history were typical. In a few typical nodular cases no examination was made.

*History of kala-azar*—In 24 cases there was a history of kala-azar and treatment, in 20 cases there was no history of kala-azar but in about half these there was a history of long continued fever and splenic enlargement. Of the 24 patients that had kala-azar 11 were treated by sodium antimony tartrate only, 3 by urea-stibamine only and 2 by both sodium antimony tartrate and urea-stibamine, in 8 instances the nature of the specific treatment was not recorded.

Our thanks are due to Major R. Knowles, I.M.S., Professor of Protozoology, Calcutta School of Tropical Medicine, in whose laboratory most of the routine examinations of the lesions were carried out and to Dr B. M. Das Gupta, his assistant, who was responsible for most of the cultural findings reported above.

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Case No. 16 before treatment



Fig 21—Case No 16 after a course of treatment

- (b) Examination of stools for total and split fats in both healthy and diseased conditions (*vide* Table II)
- (c) Examination of urine for diastatic power
- (d) Examination of blood for—
  - (i) Hæmolytic point
  - (ii) Calcium content
  - (iii) Presence of bilirubin, by Van den Bergh's test
  - (iv) Examination of lipoids in blood
- (e) Bacteriological examination of fæces in each case with special reference to intestinal flora
- (f) Experimental administration of certain intestinal hæmolytic streptococci and other non-lactose fermenting organisms to laboratory animals with a view to noting any change in the blood-forming organs
- (g) Experimental treatment of patients by autogenous vaccine and blood-forming drugs

#### RESULT OF EXAMINATION OF GASTRIC CONTENTS BY FRACTIONAL TEST MEAL METHOD

TABLE I (A)

*Examination of gastric contents of normal individuals (Indians)*  
*Average of 12 cases*

	R.	15'	30'	45'	60'
Total Acidity	06	06	07	12	16
Free HCl	04	04	.05	11	12
Total Chlorides	26	19	24	.295	.31
Mineral Chlorides	21	14	18	18	16
Active Acidity	05	05	06	115	15

N.B. R = Residual

15', 30', 45' and 60' indicate minutes after the administration of test meal

TABLE I (B)

*Examination of gastric contents of diseased individuals*  
*(Anæmia cases) Average of 14 cases*

	R	15'	30'	45'	60'
Total Acidity	03	04	08	11	12
Free HCl	02	029	056	083	09
Total Chlorides	18	19	.21	24	26
Mineral Chlorides	14	14	15	15	16
Active Acidity	04	05	06	09	104



Fig 16—Histological section of a depigmented area, commencing nodular formation. There is marked endothelial proliferation in the subpapillary layer, stained by iron haematoxylin

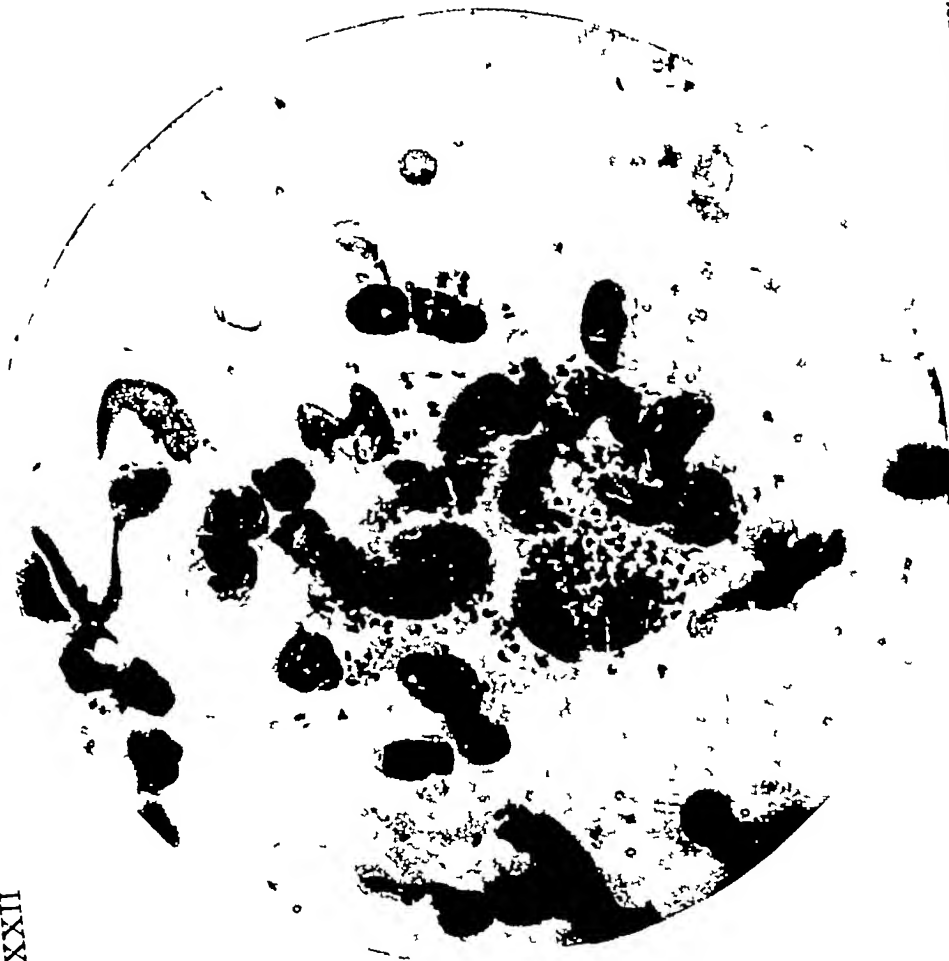


Fig 17—Smear from nodule stained by Leishman's stain showing numerous parasites

*(b) Chemical Examination of Blood*

Nature of examination	Average of 12 healthy individuals	Average of 16 diseased cases
1 Hæmolytic point	0.45 per cent NaCl	0.41 per cent NaCl
2 Van den Bergh's reaction	Indirect positive Bilirubin content 0.4 to 0.6 unit	Indirect positive Bilirubin content varying from 0.2 to 1 unit.
3 Calcium content of blood	7.5 mg per 100 c.c.	6.8 to 7 mg per 100 c.c.
4 Blood lipase (Lowen- hart's(1) method em- ployed)	0.4 to 0.3 c.c.	0.6 to 0.8 c.c.

*N.B.*—Excepting the blood lipase, it may be noted that the figures obtained are practically normal

DIASTATIC POWER OF URINE

Average of 40 normal cases	38° 15.8 d — 30° 38°
Average of 14 anæmic cases	23.2 d — 30° 38°
Average of 2 sprue cases	21.5 d — 30°

EXAMINATION OF FAT IN FÆCES

The following indices have been taken as the basis of this work —

- (a) Percentage of total fat in fæces
- (b) Percentage of neutral fat in fæces
- (c) Percentage of free fatty acid in fæces
- (d) Ratio of neutral fat to free fatty acids

I have attempted to establish a standard from the result obtained in healthy individuals and by adopting this standard to discover variations both in health and disease with a view to finding some indication which would point to the origin of the conditions under consideration

The method of examination adopted is that described by Cammidge (*The fæces of children and adults* 1914) (2)

The average age of the patients suffering from this condition is a little greater than that of the kala-azar patients, this is what one would expect

*The nature of the lesions*—Depigmented patches were noted in all the patients but in 5 instances they were not well-defined. Definite nodules were noted in 33 cases, ill-defined nodules in 4 and no nodules at all in 7 cases. There were definite xanthoma-like plaques in 3 cases and in one case this type of lesion was present but not well marked.

*Distribution of the lesions*—This is summarised in Table III

TABLE III

Depigmented patches only		Nodules or xanthoma-like lesions	No lesions
Chin	11	33	0
Cheeks	14	28	2
Nose	10	28	6
Forearms	31	7	6
Arms	29	8	7
Lips	14	17	13
Neck	27	4	13
Back	26	3	15
Brow	16	12	16
Thighs	26	1	17
Legs	22	1	21
Ears	6	14	24
Front of chest	15	5	24
Hands	10	3	31
Abdomen	7	0	37
Feet	2	1	41

*Duration of lesions*—Table IV shows the length of duration of the condition according to the type of lesion from which the patient is suffering, in 4 instances the length of duration was not recorded.

TABLE IV

Duration	Patients showing depigmented areas only	Nodular cases	Patients with xanthoma-like lesions
10 years or more			3
3 years but less than 10		5	1
2 years but less than 3		7	
1 year but less than 2	1	11	
Less than 1 year	6	6	
TOTAL	7	29	4

TABLE II (B)

Showing results of examination of 7 Indian cases with gastro-intestinal symptoms  
or anæmia with gastro-intestinal symptoms

(All on a modified Schmidt's diet as shown below )

	Percentage of total fats	Percentage of neutral fats	Percentage of free fatty acids	Ratio of neutral fats to free fatty acids	REMARKS
Case I	20 06	2 13	5 23	1 24	Anæmia, debility
Case II	38 32	5 12	19 72	1 38	Pernicious anæmia
Case III	14 40	2 50	3 05	1 12	Suspected sprue
Case IV	26 70	2 00	17 60	1 88	Dyspepsia
Case V	19 60	1 10	6 60	1 60	Chronic diarrhœa
Case VI	18 90	1 70	7 70	1 45	Chronic diarrhœa
Case VII	22 10	2 26	9 50	1 42	Suspected sprue
Average of 7 Indian cases above	22 87	2 40	9 90	1 41	For comparison with the average of 40 healthy native cases see Table II (A)
Average of 6 Euro- pean cases	27 88	3 65	13 66	1 37	All chronic diarrhœa cases

## MODIFIED SCHMIDT'S DIET

Breakfast	100 grms cream of wheat or oatmeal 60 grms toast 20 grms butter 250 c c milk.
Luncheon	Rice soup (chicken broth with rice) 100 grms green vegetables 100 grms mashed potato 60 grms toast 20 grms butter 250 c c milk
4 o'clock	250 c c milk
Dinner	150 grms chopped meat, grilled on the outside and rare in the centre 100 grms green vegetables (spinach) 100 grms mashed potato 60 grms toast 20 grms butter 250 c c milk Stewed fruit

# SOME OBSERVATIONS ON CASES OF ANÆMIA AMONGST TROOPS IN BOMBAY

BY

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THE present studies deal with an investigation into certain cases of a variety of anæmia prevalent in Bombay. The enquiry was financed by the Indian Research Fund Association. It was commenced in July 1925 but had to be closed down without being completed in April 1926. The facts which have so far been elicited are embodied in this paper.

*General features of the disease*—The onset of the disease is insidious. Almost all the cases give a history of diarrhoea simulating closely sprue, while others give a history of dyspeptic symptoms. The disease is confined mainly to Hindus who adopt a particular type of diet—deficient in animal protein. Some of the cases in the early stage exhibit marked ulceration of the tongue. The anæmia appears within six months of the disease and is generally of an aplastic type of pernicious anæmia, with a colour index varying from 1.1 to 1.7 and characteristic absence of any regenerative type of red cell. For a short period after ordinary medical treatment the patient improves a good deal and goes back to his work. An attack either of diarrhoea or malaria causes a relapse of the condition. Some cases show three or four alternating periods of the disease and ultimately die within 18 to 24 months from the onset.

The total number of cases examined was 16, out of which 14 were Indians and 2 Europeans. Of the 14, 12 were suffering from anæmia and 2 were suspected of sprue. Of the 2 Europeans, one was a case of anæmia and the other of sprue.

The various methods adopted to find out the actual seat of disease were as follows —

- (a) Examination of gastric contents for estimation of free HCl by fractional test meal. Control experiments were carried out on healthy individuals [*vide* Tables I(A) and I(B)]



My investigation was also carried out according to the basis of the former observers. It was found that there is always a preponderance of putrefactive or proteolytic type of organism in almost all the cases. Considering that almost all the cases showed symptoms of diarrhoea at one stage or another, the question arises whether it is not possible, that the toxins of these proteolytic organisms do exert an injurious effect on the red cells of individuals causing an enormous destruction thereof, specially so when there is a deficiency of HCl in the stomach. The fact that two cases did improve after a course of vaccine treatment also supports this view. The injurious effects of the injection of these organisms into laboratory animals are shown below.

Effect of 0.25 c.c. of a 24-hour broth culture of non-lactose fermenting bacilli isolated from the stool of Case Nos. 2 and 8.

Rabbit No. 1—It developed septicaemia and died within 18 hours after injection. The organism was isolated from heart, liver, spleen and peritoneum in pure culture.

Rabbit No. 2—A much smaller dose was used, but the rabbit died six days after inoculation. The organism was isolated in pure culture from liver and peritoneum. Section of liver showed fatty degeneration, those of spleen and kidney showed intense congestion and hæmorrhages.

Character of the non-lactose fermenting organisms. It is gram-negative, sluggishly motile. It ferments glucose, inositol and arabinose with the production of acid and gas. It does not ferment lactose, saccharose, inulin and raffinose. The organism does not agglutinate with high titre sera against Typhoid, Para A, B, and C, or organisms of the Dysentery or Aertrycke Group.

The experiment on this line could not be carried out any further.

#### EFFECT OF HÆMOLYTIC STREPTOCOCCI ON ANIMALS

*On Rabbit*—No action was noted on injection of 0.25 c.c. of saline suspension of a 24-hour blood agar culture and hence the dose was increased—but the rabbit died after three days. Post-mortem appearance showed congestion of all the organs which was also confirmed microscopically.

*On Guinea-pig*—A healthy guinea-pig was inoculated with 0.25 c.c. of the suspension and this too died after five days. All the organs were congested. Bone marrow was dark red, rather gelatinous. Section of bone marrow showed—hyperplastic tissue—new formation of myelocytes and myeloblasts. New formed lymphoid tissue was present and few megaloblasts seen. Further experiments on these lines should be carried out before coming to a definite conclusion as to the rôle these organisms play in producing anæmia. Unfortunately this could not be done owing to the premature closure of the work.

It may be noted that the non-lactose fermenting organism and hæmolytic streptococci which were isolated from the stool did not agglutinate with the patient's own serum. In spite of this fact an autogenous vaccine was prepared and 2 courses of 8 doses were administered in 2 cases. Both the cases improved remarkably and were discharged to duty. They are quite well now—six months after discharge.

*Average (sprue cases) of 3 cases*

	R	15'	30'	45'	60'
Total Acidity	06	06	08	08	08
Free HCl	03	04	06	06	05
Total Chlorides	20	19	23	25	26
Mineral Chlorides	15	14	16	17	18
Active Acidity	05	05	07	03	08

NB R = Residual

15', 30', 45' and 60' indicate minutes after the administration of test meal

From the above tables it will be seen that in almost all the diseased cases there is a deficiency of free HCl in the gastric juice. It may be noted in this connection that the normal percentage of HCl found in healthy Indians is below the European standard.

## EXAMINATION OF BLOOD

(a) *Total Counts, Differential Counts, Indices, etc*

	Total red blood corpuscles	Total leucocytes	Hæmoglobin (per cent)	Colour Index	DIFFERENTIAL COUNT			PERCENTAGES	
					Polys	Lymphoc.	L Mono	Eosin-op	Basop
Case No 1	1,680,000	4,500	45	1.4	49	43	5	2	1
" " 2	3,475,000	5,000	55	1.8	46	47	5	2	
" " 3	3,600,000	7,500	90	1.25	72	24	2	2	
" " 4	1,602,500	3,750	45	1.4					
" " 5	1,900,000	7,200	60	1.3	50	31	15	4	
" " 6	1,662,000	3,485	45	1.4					
" " 7	3,650,000	5,625	65	0.9	60	34	4.5	1.5	
" " 8	3,138,000	5,056	60	1.05	64	28	6	1	1
" " 9	1,587,500	3,437	45	1.5	56	36	3	5	
" " 10	3,700,000	5,625	65	.88	57	33	7	2	1
" " 11	5,175,000	8,750	70	.7	57	35	4	3	1
" " 12	2,700,000	4,687	55	1.3	55	39	5	1	
" " 13	4,200,000	11,500	80	.95	53	38	8	1	
" " 14	3,190,000	6,825	68	1.06	61	31	5	2	1

Note—(1) All these cases showed marked poikilocytosis, and polychromatophilia. Excepting two cases none showed any regenerative forms of red cell.

(2) The total red cells of Case Nos 6 and 9 went down to 1,000,000 per cmm when 10 ozs of blood were transfused which improved their general condition. Case No 6, has since been invalided to England.

(3) None of the cases showed any eosinophilia.

(4) Case Nos 11 and 13 are suspected sprue cases.

experiments should be carried out before any definite conclusion can be arrived at in this matter

11 Two cases improved after administration of autogenous vaccine of intestinal organisms, hence treatment on this line may be helpful

12 No improvement was noticed in suspected sprue cases after administration of calcium and parathyroid extract

13 There is slight diminution of blood lipoids in almost all the cases, showing deficiency of absorption fats

14 Finally until a large number of cases have been examined, no definite conclusion can be arrived at as to whether the anaemia cases noted above, are primary or secondary in origin. It may be noted that in all cases repeated examination of the stools by Clayton Lane's method failed to show any ova of ankylostoma

In conclusion I beg to thank Colonel R. H. Lloyd, A. D. M. S., Bombay District, for his kindly permitting me to concentrate all these cases at one place for investigation and to Lieut. G. B. Gollerkeri, I. M. S. (T. C.), for his valuable assistance in treating these cases according to my suggestion and to Captain S. L. Bhatia, M. D., M. C., I. M. S., Dean of Grant Medical College, Bombay, for his kindly lending me various apparatus, re-agents, etc., from time to time to carry out the experiments

Lastly, I must acknowledge my indebtedness to Assistant Surgeon R. D. Wilson, I. M. D., and to Mr. T. G. Paymaster, M. B., B. S., for their kind assistance in helping me to carry out these investigations

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- |                                     |   |
|-------------------------------------|---|
| (1) LOWENHART (1902)                | <i>Amer Jour Physiology</i> , Vol VI, 331             |
| (2) CAMMIDGE (1914)                 | <i>The Faeces of Children and Adults</i>              |
| (3) CUSHING and LIVINGWOOD (1900)   | <i>Johns Hopkins Hospital Reports</i> , Vol IX, p 543 |
| (4) MORRIS, PORTER and MEYER (1919) | <i>Jour Infect Dis</i> , Vol XXV, pp 349—377          |

TABLE II (A)

Showing results of the examination of 40 'healthy' Indians on varying hospital diets

	Percentage of total fats	Percentage of neutral fats	Percentage of free fatty acids	Ratio of neutral fats to fatty acids	REMARKS
Average of 40 cases	10.89	1.38	3.98	1 : 2.9	Cambridge gives the ratio of neutral fats to free fatty acids as 1 : 10 in health
Average of 10 cases with highest percentage of total fats	16.47	1.55	6.42	1 : 4.1	
Average of 10 cases with lowest percentage of total fats	7.78	1.51	2.82	1 : 1.9	
Average of 10 cases with highest percentage of neutral fats	11.45	1.78	4.50	1 : 2.5	
Average of 10 cases with lowest percentage of neutral fats	10.83	0.93	4.68	1 : 5.0	
Average of 10 cases with highest percentage of free fatty acids	13.35	1.41	7.34	1 : 5.2	
Average of 10 cases with lowest percentage of free fatty acids	8.72	1.65	2.24	1 : 1.4	
Average of 10 cases with highest neutral fat, free fatty acid ratio	14.50	1.17	6.70	1 : 5.7	
Average of 10 cases with lowest neutral fat, free fatty acid ratio	8.79	1.67	2.24	1 : 1.3	

the quinine derivative optochin, which is probably the nearest approach to an effective chemotherapeutic agent in septicæmia or general infectious conditions, can be given in such quantities as to render a rabbit's blood bactericidal to pneumococci, but yet clinically does not influence the course of a pneumococcal infection in a rabbit. Again, trypan blue, and numerous compounds of trypan red and of Niagara blue have a strong bactericidal action upon tubercle bacilli *in vitro*, and also produce a selective staining on the bacilli *in vivo*, but none of these compounds inhibits the growth of the tubercle bacillus in the living animal.

With such instances in mind one might feel inclined to question the soundness of our purpose to study the specific therapy of plague, a bactericidal infection which progresses so rapidly as to necessitate the use of a quick-acting drug. Still we persist in our belief that patient, systematic exploration by routes already mapped out in the field of chemotherapy must lead to results of major importance as regards both the practical and the theoretical sides of specific therapy.

In chemotherapeutic work experiments *in vitro* are not without value for purposes of general orientation, and they may help in (1) the correlation of chemical constitution and bactericidal power, and (2) the selection of germicides likely to be efficacious as internal disinfectants. This is the twofold object of our enquiry.

We have accordingly decided to base the selection of the drugs to be tested *in vivo* upon their bactericidal action on *B. pestis* as determined by experiments *in vitro*.

Our intention is to study known drugs first and then, if results open a new vista, to continue with synthetic products. This paper deals with the commoner phenols and some of their derivatives and is restricted to results obtained *in vitro*.

The knowledge of the comparative effects of various antiseptic or bactericidal substances is still inconclusive. The method whereby their efficacy is compared to that of phenol is not in any manner as reliable as many seem to believe. As workers find it difficult to check one another for the phenol coefficient on phenol compounds, it is logical to suppose that more trouble would be experienced with non-phenolic compounds. The fact is that phenol coefficients for non-phenolic compounds are not reliable.

We have, therefore, adopted an alternative method which consists in cultivating the organisms in broth containing definite amounts of the disinfectant, and observing the exact concentration necessary to prevent development of the organism. To this effect a 24 hours' plague culture in broth was used. 5 c.c. of a solution of the drug were added to 5 c.c. of the broth culture and the whole allowed to stand at room temperature (30°—31°) for 15 minutes, 1 hour, 2 hours, 24 hours, 48 hours, when sub-cultures were made on agar slopes. The tubes were incubated for 3 days at room temperature and the presence or absence of growth observed.

Observation showed that under the conditions *B. pestis* was not only inhibited, but was actually destroyed, so that the technique may be considered as measuring bactericidal power. As a general result we have noted that though most drugs exert an appreciable germicidal action in 15 minutes, still their maximum efficacy is not attained until after a period of 24 hours. We have

TABLE III (C)

Showing results of the examination of 42 'healthy' Indians on certain diets

			Percentage of total fats	Percentage of neutral fats	Percentage of free fatty acids	Ratio of neutral fats to free fatty acids	REMARKS
No 1	Diet	22 cases	10.55	1.38	3.81	1 : 2.8	
No 2	Diet	7 cases	9.84	1.38	4.27	1 : 3.2	
No 3	Diet	3 cases	17.03	1.56	4.82	1 : 3.1	
Unknown diet		10 cases	11.00	1.26	4.91	1 : 3.9	

No 1 DIET		No 2 DIET		No 3 DIET	
Atta	1 lb	Atta	1 lb	Arrowroot	2 ozs
Dal	3 ozs				
Ghi	2 ozs	Ghi	2 ozs		
Mutton	2 ozs	Mutton	8 ozs		
Vegetables	4 ozs	Vegetables	8 ozs		
Bread	2 ozs	Bread	4 ozs		
Sugar	2 ozs	Sugar	3 ozs	Sugar	4 ozs
Milk	2 ozs	Milk	2 lbs	Milk	1 lb

By reference to the tables it will be seen that certain factors have been taken as guides, e.g., relative percentage of total fat, neutral fat and of free fatty acids and the neutral fat—free fatty acid ratio. How far these figures will approximate to a fixed standard can only be determined by a large series of analyses. Variation is almost inevitable in view of the play of other factors than those solely concerned in the metabolism of fat—rapidity of passage and intestinal bacterial action need only be mentioned to illustrate this point.

#### BACTERIOLOGICAL EXAMINATION OF FÆCES

Intestinal flora have been classified in many ways by various investigators. Cushing and Livingood(3) divided them into two groups (1900)—

(1) Permanent or obligatory

(2) Transient or facultative. The first being always present the second is unable to flourish in the presence of the first, unless some pathological lesion of the mucosa is present.

Torrey (1919) reclassified the organisms into 3 groups—

(1) Fermentative, i.e., *B. welchii*, *Streptococci*

(2) Putrefactive, e.g., *B. sporogenes*, *B. proteus*, *B. pyocyaneus*

(3) *B. coli* group lying midway between the two groups

Subsequently Morris, Porter and Meyer(4) also divided the flora into 3 groups—

(1) Fermentative or saccharolytic

(2) Putrefactive or proteolytic

(3) Facultative or normal

The entrance of the hydroxyl group into benzene, with the formation of phenol, causes a great increase in antiseptic properties. Phenol, or carbolic acid, was the first antiseptic to be widely used. The introduction of alkyl groups into the nucleus increases the antiseptic properties, as shown by the three isomeric cresols which are better antiseptics than phenol.

The homologous phenols, however, are much less soluble in water than phenol itself, and the same is true of very many of the substitution products. For this reason we have employed in this enquiry solutions made up by dissolving the substances in just enough 5-6 per cent aqueous caustic soda and diluting to the required concentration. Failing this, the sodium compound was prepared, and its solution in water used.

As shown by Table I —

1. Solution in alkali either depresses or intensifies the bactericidal power of monohydric phenols, without any apparent cause.

2. The fall is most marked with thymol.

3. Whereas the bactericidal power of thymol (3-methyl-6-isopropyl phenol) is greatly depressed in alkali solution, that of its isomer, carvacrol (2-methyl-5-isopropyl phenol) is intensified.

4. The entrance of a methyl group in the nucleus of sodium phenolate increases the bactericidal value. The further entrance of an isopropyl group increases the bactericidal power of 1,2-methylhydroxybenzene, but depresses that of 1,3-methylhydroxybenzene.

As may be seen from Table II —

1. Of the three dihydroxybenzenes resorcinol is the least toxic to *B. pestis*, and the bactericidal power of the sodium compound is practically the same as that of the free phenol.

2. The entrance of a methyl group in the molecule of resorcinol (orcinol) greatly lowers the bactericidal value of the free phenol, but does not affect the sodium compound to any great extent.

3. Compared with resorcinol its two isomers, catechol and quinol, are highly toxic to *B. pestis*, especially in alkali solution.

4. The bactericidal value of quinol is greatly lowered by the entrance of a methyl group in its molecule (toluhydroquinone). The further entrance of an isopropyl group considerably depresses the bactericidal power, and thymohydroquinone is more than 2,000 times less potent than quinol.

5. The trihydric phenols, pyrogallol and phloroglucinol, have a comparatively low bactericidal power.

6. In general the bactericidal power of polyhydric phenols is intensified in alkali solution.

Table III shows that —

1. Etherification lowers the antiseptic value of the dihydric phenols,

2. The entrance of a propenyl radical, whether allyl (eugenol) or isoallyl (isoeugenol), in the molecule of guaiacol enhances the bactericidal power of that compound.

## EFFECT OF VARIOUS DRUGS AND TRANSFUSION OF BLOOD

Iron and arsenic ampoules kindly supplied free of charge by the Bengal Biochemical Laboratories, Calcutta, were administered in some of the cases. Most of them showed temporary benefit. In three cases 8 ozs of blood were transfused on two occasions. One European case improved very much. Two Indian cases, however, improved for a time but did not keep up the improvement. Calcium and parathyroid treatment was administered in all suspected cases of sprue—but with no benefit.

## CONCLUSION

1 There is a marked deficiency of free HCl on gastric analysis in almost all the cases—it may be noted that the standard for Indians is much lower than that of Europeans.

2 The estimation of neutral fat and free fatty acids in faeces showed altogether a different ratio than that hitherto described. Cammidge gives the normal ratio as 1 : 10, whereas the average of 40 healthy individuals (Indians) showed 1 : 2.9. In all diseased conditions, there is disturbance of this ratio. In one case the ratio was as high as 1 : 8.8, but in almost all the cases there is an increase of free fatty acid. I think this increase is mainly due to the action of intestinal bacteria as when antiseptics such as Dimol, were administered, the increase of free fatty acid was not so noticeable. Other factors, e.g., rapidity of passage, also alter this ratio.

3 The excretion of total fat was not high—thus differing from sprue where fats form more than 40 per cent of the total weight of dried faeces.

4 None of the anaemia cases showed marked regenerative type of red cells—thus showing that they are mostly aplastic in nature.

5 Van den Bergh's test gave a positive indirect reaction with slight increase of bilirubin content in comparison with that of healthy Indians showing that the anaemias are more of a haemolytic nature.

6 The diastatic power of urine was normal—thus excluding pancreatic disease.

7 As shown by the fragility test, the red cells did not show any diminished resistance in comparison with the red cells of healthy individuals.

8 Although the calcium content in blood is lower than the European figure, yet there is not much deficiency in comparison with those of healthy Indians.

9 Bacteriological examination of the stools revealed the presence of a large number of putrefactive and fermentative organisms and the injection of these organisms into laboratory animals showed that they are virulent in type. This experiment could not be carried out fully owing to the premature closure of this investigation, but it is hoped that other observers will be able to throw further light on the subject.

10 In my opinion it is a mistake to discard organisms isolated from the stool, simply because these do not agglutinate with patient's own sera. These organisms may not normally have any effect on the individual concerned but they may be active if some suitable nidus is provided. However, further



TABLE III  
Ethers of Dihydric Phenols

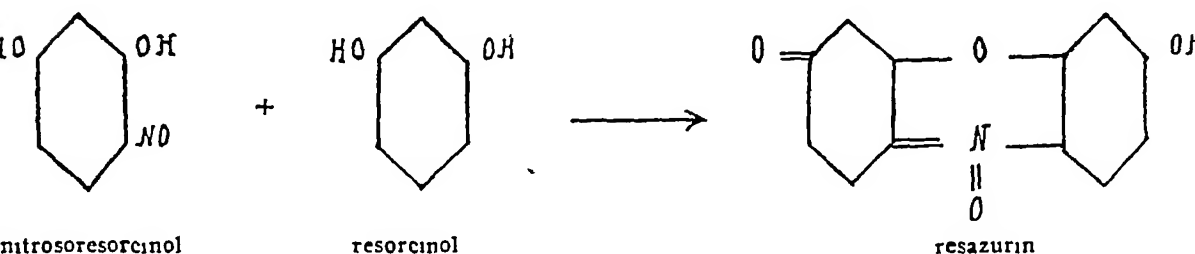
				15 minutes contact	24 hours contact
guaiacol	$C_6H_4$	OH (1)		1 400	1 800
		$OCH_3$ (2)			
resorcinmonomethylether	$C_6H_4$	OH (1)		1 150	1 600
		$OCH_3$ (3)			
hydroquinonemonomethylether	$C_6H_4$	OH (1)		1 480	1 960
		$OCH_3$ (4)			
eugenol	$C_6H_3$	OH (1)		1 2000	1 4000
		$OCH_3$ (2) $CH_2-CH=CH_2$ (4)			
isoeugenol	$C_6H_3$	OH (1)		1 1500	1 2500
		$OCH_3$ (2) $CH=CH-CH_3$ (4)			

### SUBSTITUTED PHENOLS

The hydroxyl group has a marked effect on the chemical character of the aromatic radicle, causing certain of the hydrogen atoms attached to nuclear carbon atoms to be more amenable to substitution by other atoms or groups. Thus benzene can be brominated only by dry bromine in presence of a hydrogen carrier, but phenol, on treatment with bromine water, at once yields 2, 4, 6-tribromophenol. Similarly, nitrophenols are produced by treatment of phenol even with dilute nitric acid. Furthermore, phenol sulphonates more readily than benzene.

We have investigated various derivatives of phenol, resorcinol, quinol, and the naphthols.

Among the derivatives of resorcinol will be found resazurine. This compound, which has been obtained synthetically by oxidising a mixture of nitrosoresorcinol and resorcinol, is an *oxazone* and belongs to the group of dyestuffs derived from quinone-imide —



# THE BACTERICIDAL ACTION OF THE COMMONER PHENOLS AND OF SOME OF THEIR DERIVATIVES ON *BACILLUS PESTIS*

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SINCE the days of Koch a number of attempts have been made to find some drug which, when given intravenously, would disinfect the blood in cases of bacterial infection. Although favourable results have been reported to have followed the intravenous injection of iodine and eusol, yet, at present, the only example of successful treatment being applied to bacteria living in the tissues of the body is that of the unsaturated fatty acids of chaulmoogra oil used in the treatment of leprosy.

On the other hand we know several specific drugs which cause the destruction of protozoa within the body, such as mercury, quinine, emetine, antimony tartrate, and the organic arsenicals.

The introduction of organic arsenic compounds for the treatment of syphilis and of other diseases caused by spirillæ, spirochætes, and trypanosomes was the result of a prolonged research by Ehrlich and his co-workers. The research was conducted with the definite purpose of discovering a compound which would have a maximal destructive action upon the parasites and a minimal toxic action upon the host. It culminated in the discovery of salvarsan.

The discovery of salvarsan has stimulated investigation, the object of which has been to find chemicals with a similar specific action in bacterial infections. So far, however, the search cannot be considered successful because of the fact that substances which *a priori* appear to possess all the properties necessary for a specific disinfectant action fail in practice to exert any such effect. For example,

4 The acetyl group lowers the bactericidal power, while the salicyl group increases it

5 The aldehyde derivative of resorcinol is more powerful than resorcinol, while the aldehyde derivative of guaiacol is less active than guaiacol

6 Resazurin is seven times as active as resorcinol

TABLE V  
*Resorcinol Derivatives*

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact	24 hours contact.
Resorcinol	1 800	1 800
4- nitrosoresorcinol	1 200	1 800
2 4- dinitrosoresorcinol	1 400	1 1600
2- nitroresorcinol	1 300	1 2400
2 4- dinitroresorcinol	1 300	1 300
2 4 6- trinitroresorcinol	nil	nil
4 6- diaminoresorcinol	1 2400	1 3000
4 6- diacetylresorcinol	1 300	1 600
4 6- disalicylresorcinol	1 2400	1 4800
dimethylhydroresorcinol	1 300	1 300
diazoresorcinol (resazurin)	1 4800	1 6000
resorcyaldehyde (4 - al)	1 300	1 2400
Guaiacol	1 400	1 800
vanillin (guaiacolaldehyde)	1 200	1 200

Table VI shows that substitution in the molecule of quinol leads to the formation of much less active substances

TABLE VI  
*Quinol Derivatives*

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact	24 hours contact.
quinol	1 308000	1 432000
tetrachlorhydroquinone	1 1200	1 2000
nitrohydroquinone	1 1000	1 6000
triacetylhydroxyhydroquinone	1 1600	1 2400

recorded the results obtained after a contact of 15 minutes as they are of value in the selection of a germicide likely to be efficacious against *B. pestis*, and also the results obtained after a contact of 24 hours as these furnish a reliable standard for the discussion of the correlation between chemical constitution and bactericidal power

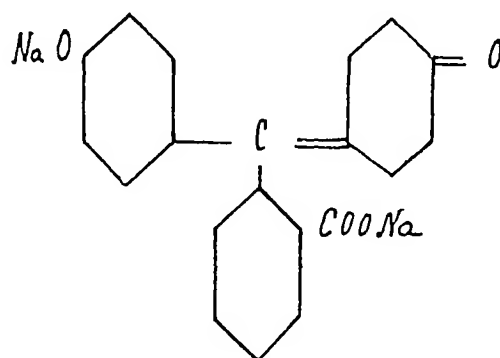
### PHENOLS

Phenols are hydroxy-compounds of the aromatic series in which the hydroxyl group is attached to the nucleus. They are classed as mono-, di-, tri-hydric, etc., according to the number of hydroxyls present. Carbolic acid and the three isomeric cresols or hydroxytoluenes are monohydric, resorcinol and its isomers are dihydric, and phloroglucinol is a trihydric phenol.

TABLE I  
*Monohydric Phenols*

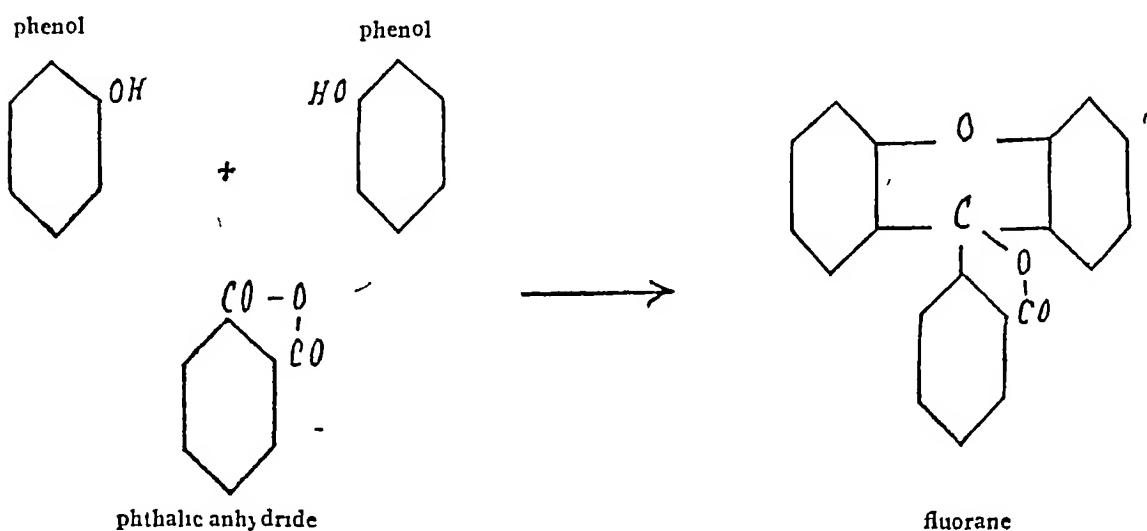
		BACTERICIDAL CONCENTRATIONS			
		15 MINUTES CONTACT		24 HOURS CONTACT	
		Free Phenol	Na compound	Free Phenol	Na compound
Phenol	$C_6H_5$ OH	1 400	1 600	1 800	1 600
o-cresol	$C_6H_4$ OH (1)	1 400	1 800	1 2400	1 1000
	$CH_3$ (2)				
m-cresol	$C_6H_4$ OH (1)	1 400	1 3600	1 800	1 3600
	$CH_3$ (3)				
p-cresol	$C_6H_4$ OH (1)	1 200	1 2400	1 400	1 2400
	$CH_3$ (4)				
isobutyl phenol	$C_6H_4$ OH (1) $C_4H_9$ (4)		1 2800		1 4800
isoamyl phenol	$C_6H_4$ OH (1) $C_5H_{11}$ (4)		1 3200		1 4800
thymol	$C_6H_3$ OH (1) $CH_3$ (3) $C_2H_5$ (6)	1 4000	1 200	1 8000	1 300
carvacrol	$C_6H_3$ OH (1) $CH_3$ (2) $C_2H_5$ (5)	1 4000	1 9600	1 6000	1 11200
alpha-naphthol	$C_{10}H_7$ OH (1)	1 4000	1 1200	1 4000	1 1600
beta-naphthol	$C_{10}H_7$ OH (2)	1 2000	1 1200	1 6000	1 2400

Phenolphthalein, which is colourless, is thus a lactone, or internal ester, and, like other esters, can be hydrolysed by means of alkali. This hydrolysis is readily effected, and takes place instantly on addition of alkali, with formation of a coloured metallic salt of quinonoid structure



sodium salt of phenolphthalein

In the preparation of phenolphthalein a by-product, *fluorane*, insoluble in alkalis is formed. This substance results from the condensation of two molecules of phenol with one of phthalic anhydride in such a way that the two phenol residues are united at the *ortho*-positions to the phthalic-anhydride residue, and not at the *para*-positions, as in phenolphthalein

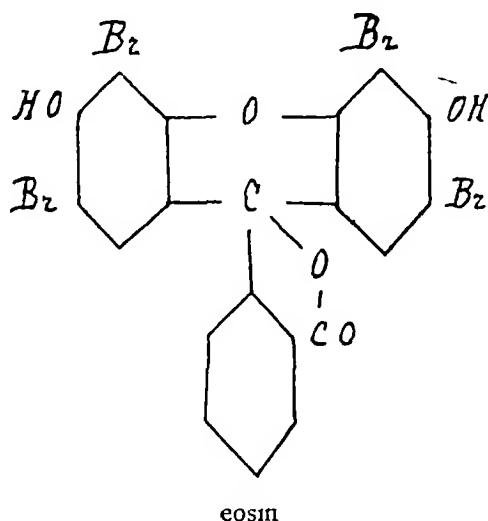


Fluorane is the mother substance of a large number of dyes. In order the better to understand their constitution and to avoid the use of meaningless

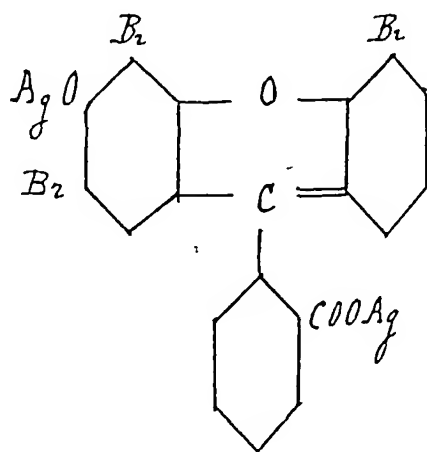
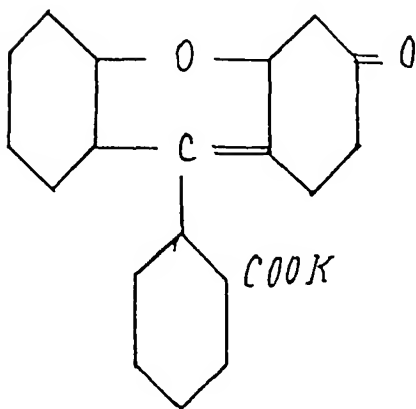
TABLE II  
Polyhydric Phenols

			BACTERICIDAL CONCENTRATIONS			
			15 MINUTES CONTACT		24 HOURS CONTACT	
			Free Phenol	Na compound	Free Phenol	Na compound
A Dihydric Phenols						
catechol	$C_6H_4$	OH (1) OH (2)	1 6400	1 24000	1 32000	1 48000
resorcinol	$C_6H_4$	OH (1) OH (3)	1 800	1 800	1 1200	1 800
quinol (hydroquinone)	$C_6H_4$	OH (1) OH (4)	1 16000	1 308000	1 16000	1 432000
orcinol	$C_6H_4$	OH (1) OH (3) CH <sub>3</sub> (5)	1 150	1 600	1 150	1 1000
toluhydroquinone	$C_6H_4$	OH (1) OH (4) CH <sub>3</sub> (5)	1 1000	1 4800	1 4000	1 14400
thymohydroquinone	$C_6H_4$	OH (1) OH (4) CH (5) C <sub>2</sub> H <sub>5</sub> (2)			1 150	1 200
naphthoresorcin	$C_{10}H_6$	OH (a) OH (b)	1 500	1 400	1 1000	1 600
phenoresorcin			1 600	1 300	1 1200	1 600
B Trihydric Phenols						
pyrogallol	$C_6H_3$	OH (1) OH (2) OH (3)	1 200	1 400	1 600	1 1000
phloroglucinol	$C_6H_3$	OH (1) OH (3) OH (5)	1 150	1 200	1 300	1 600

Thus fluorescein, or resorcinolphthalein, is 3 6—dihydroxyfluorane. On treatment with bromine, fluorescein yields eosin, that is 2 4 5 7—tetrabromo—fluorescein or 2 4 5 7—tetrabromo—3 6—dihydroxyfluorane.



In general behaviour towards alkalis fluorescein and its derivatives resemble phenolphthalein and yield coloured metallic salts of quinonoid structure.



As may be seen from Table IV —

1 The entrance of chlorine or bromine into the nucleus of phenol causes an increase in bactericidal power — 2 4 6—trichlorphenol is sixteen times more powerful than phenol

2 Nitration does not alter the bactericidal value of phenol to any great extent

3 The entrance of the amino group increases the bactericidal power — 2 4—diaminophenol is twenty times as active as phenol, 2—amino—4 6—dinitrophenol (picraminic acid) is five times as active as 2 4 6—trinitrophenol (picric acid)

4 The entrance of an acid grouping, whether the carboxyl radicle—COOH or the sulphonic group—SO<sub>2</sub> OH, depresses the bactericidal power of phenol

TABLE IV  
*Phenol Derivatives*

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact	24 hours contact
Phenol	1 600	1 600
2 4 6- trichlorphenol	1 2000	1 10000
2 4 6- tribromphenol	1 1500	1 3000
0- nitrophenol	1 200	1 300
p- nitrophenol	1 300	1 1000
2 4- dinitrophenol	1 400	1 800
2 4 6- trinitrophenol (picric acid)	1 250	1 400
2-amino-4 6- dinitrophenol (picraminic acid)	1 600	1 2000
0- aminophenol	1 800	1 3200
p- aminophenol	1 1200	1 2400
2 4- diaminophenol	1 9600	1 12800
salicylic acid	1 100	1 200
anisic acid	1 200	1 300
p- phenolsulphonic acid	1 300	1 400
salicylsulphonic acid	1 300	1 400

Table V shows that —

1 The nitroso derivatives act more slowly than resorcinol and the dinitroso compound is twice as active as the mononitroso derivative

2 The mononitro derivative is three times as active as resorcinol, while dinitro derivative is less powerful, and the trinitro compound is inert

3 The entrance of the amino radicle increases the bactericidal power



TABLE VIII

*Phthaleins of monohydric Phenols*

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact	24 hours contact.
Phenol	1 600	1 600
phenolphthalein	1 200	1 200
0-cresolphthalein (= 4 5-dimethylphenolphthalein)	1 1200	1 1600
thymolphthalein (= 1 8-dimethyl-4 5-di-isopropyl-phenolphthalein)	1 1600	1 2400
2 4 5 7- tetrachlorophenolphthalein	1 800	1 800
diacetylphenolphthalein (= phenolphthalein diacetate)	1 2400	1 3600
alphanaphtholphthalein	1 1600	1 2000

As shown by Table IX the phthaleins of resorcinol and pyrogallol are less active than the corresponding phenols

TABLE IX

*Phthaleins of polyhydric Phenols*

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact	24 hours contact.
Resorcinol	1 800	1 800
resorcinolphthalein (fluorescein)	1 150	1 150
Pyrogallol	1 400	1 1000
pyrogallolphthalein (gallein)	1 100	1 200

As shown by Table X —

- 1 Reduction of fluorescein (fluorescein) enhances its bactericidal value
- 2 The dimethyl compound (orcinolphthalein) is more powerful than fluorescein
- 3 The tetrabromo derivative is completely inert, while its dimethyl homologue is as active as orcinolphthalein

As may be seen from Table VII —

1 The entrance of the carboxyl radicle in the molecule of a naphthol tends to lower the bactericidal power

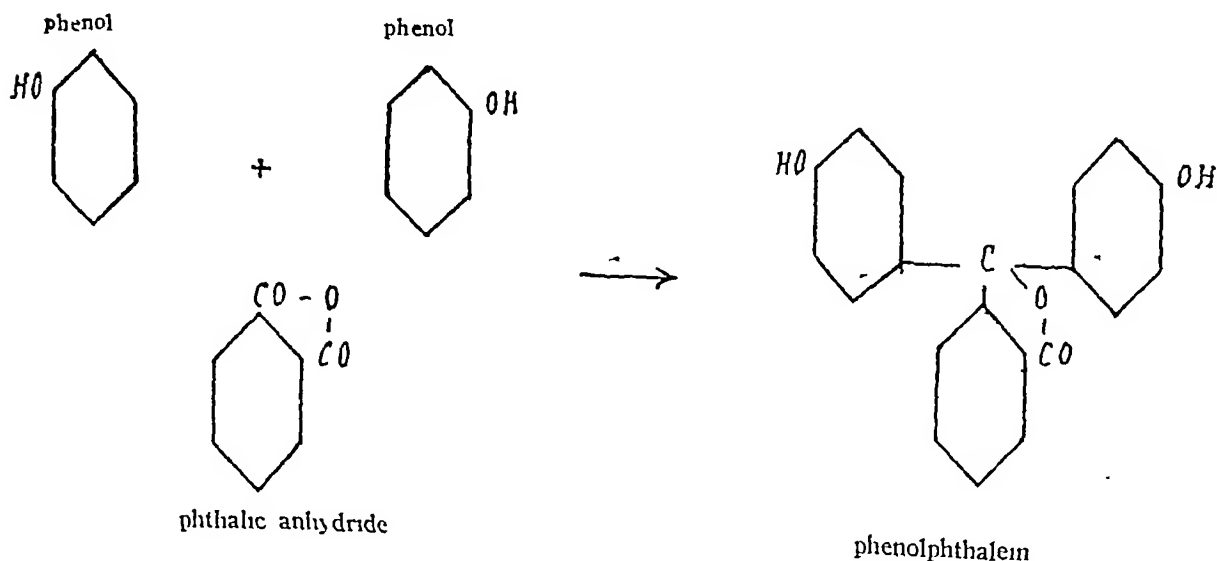
2 The entrance of the nitroso radicle in the molecule of betanaphthol makes this compound sixteen times more active

TABLE VII  
*Derivatives of the Naphthols*

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact.	24 hours contact.
alphanaphthol	1 1200	1 1600
alpha-hydroxynaphthalic acid	1 600	1 1600
betanaphthol	1 1200	1 2400
alphanitroso-betanaphthol	1, 3200	1 25600
beta-hydroxynaphthalic acid	1 400	1 600

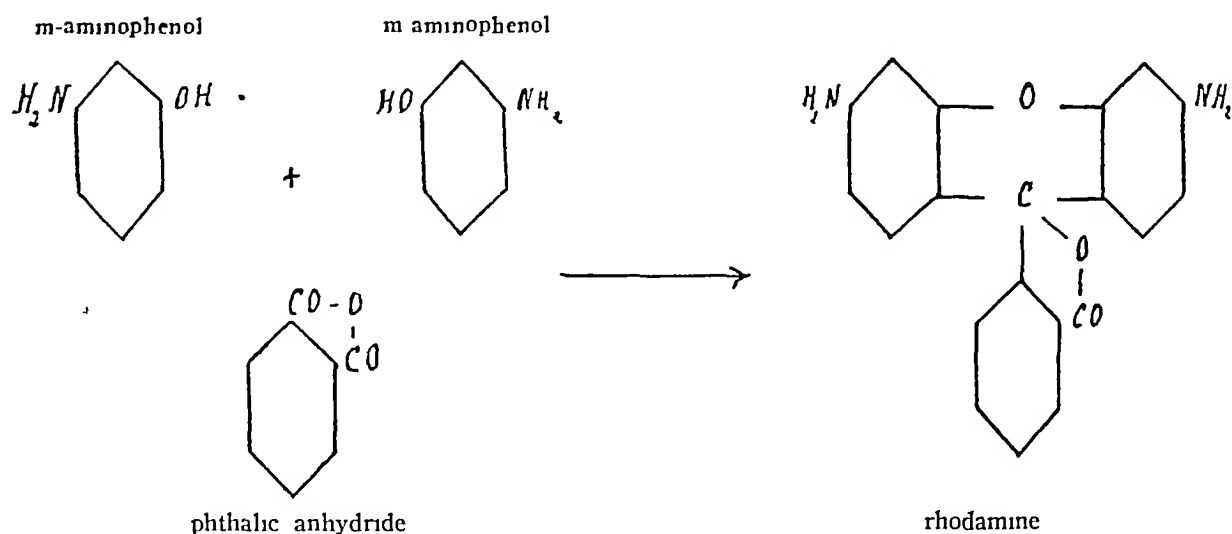
### PHTHALEINS

Phthaleins are compounds obtained by the condensation of two molecules of a phenol with one of phthalic anhydride. For example with phenol, phenolphthalein, the well-known indicator, is obtained —



## RHODAMINES

The rhodamines are the phthaleins of m-aminophenol. The simplest rhodamine is formed when m-aminophenol hydrochloride and phthalic anhydride are heated to  $190^{\circ}$  with concentrated sulphuric acid —



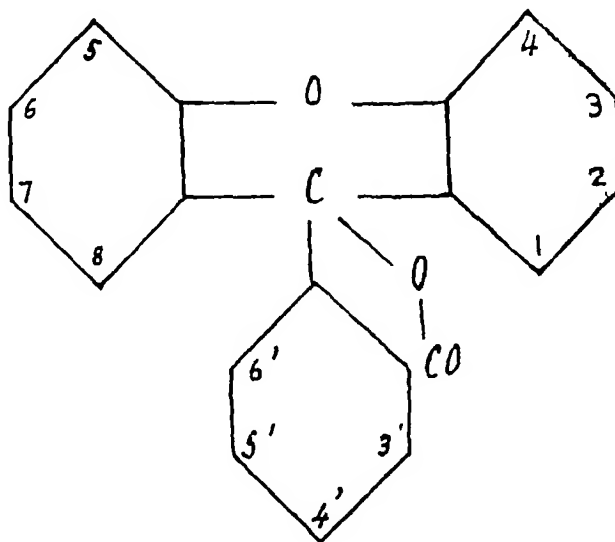
Alkyl rhodamines are produced when rhodamine hydrochloride is heated with alkyl iodides, or, better still, by condensing alkyl-m-aminophenol with phthalic anhydride

Table XI shows that rhodamines are little toxic to *B. pestis*

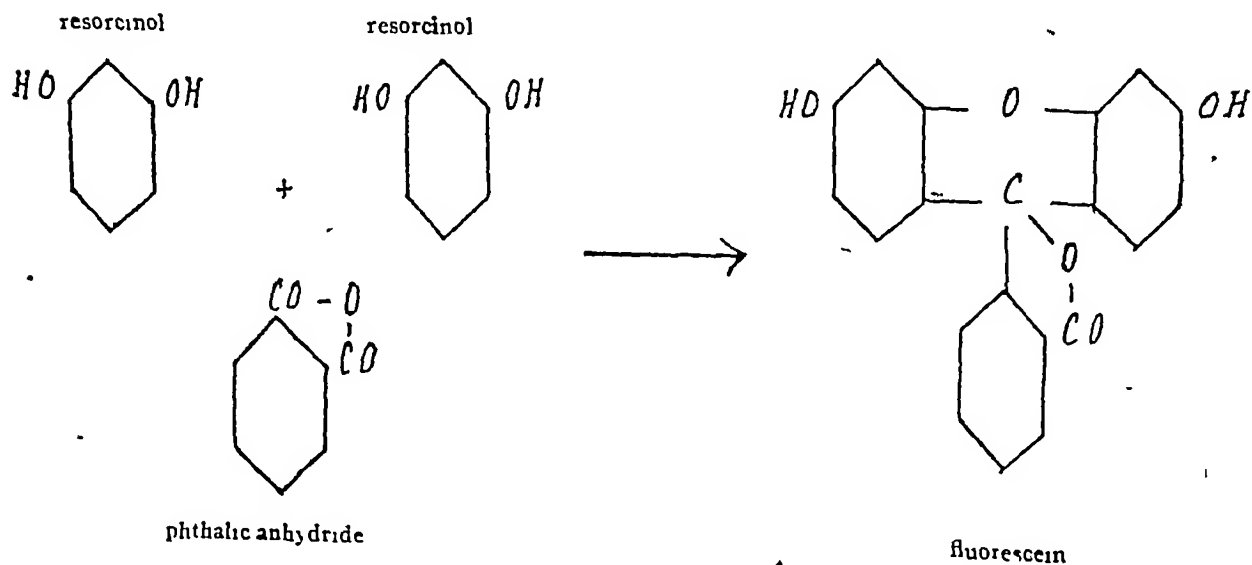
TABLE XI  
Rhodamines

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact	24 hours contact.
tetraethyl-3,6-diaminofluorane	no action	1:150
3-phenylamino-6-phenylaminometasulphonic-fluorane	no action	no action
3,6-dichloro-3-paraethoxyphenylamino-6-para-ethoxy-phenylaminometasulphonic-fluorane	1:100	1:400

commercial names we will adopt the following scheme of numbering for fluorane and its derivatives —



Resorcinol couples with phthalic anhydride, the point of attachment being ortho to one and para to the other of the hydroxyl groups. At the same time water is eliminated from the ortho hydroxyl groups and fluorescein is produced



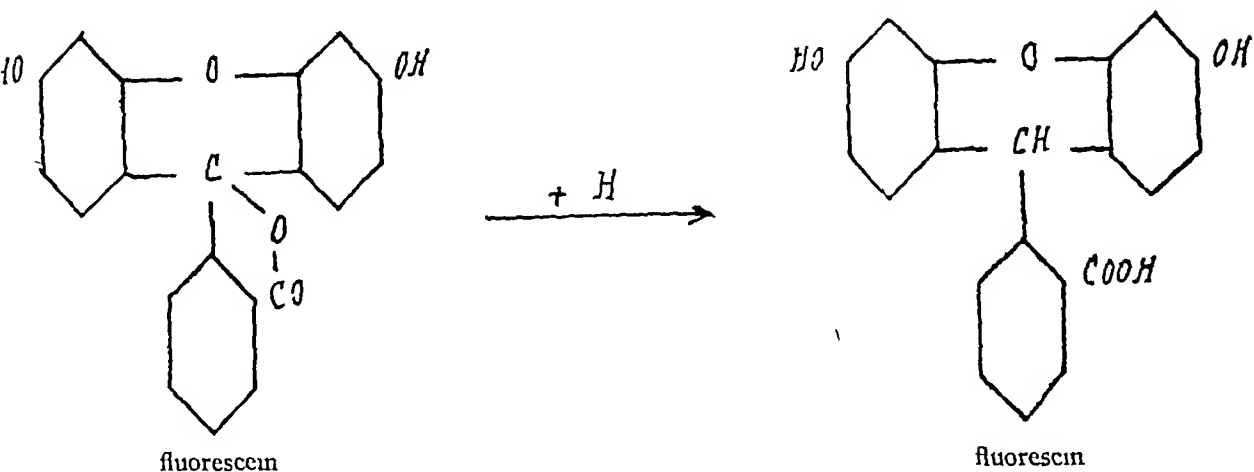
The substances studied fall within four main groups —(1) the phenols, (2) derivatives of phenols, (3) the phthaleins, (4) derivatives of fluorescein

Most of the compounds are insoluble in water, but dissolve readily in alkalis. Solution in alkali either depresses or intensifies the bactericidal power of monohydric phenols, without any apparent cause, but generally enhances that of the polyhydric phenols

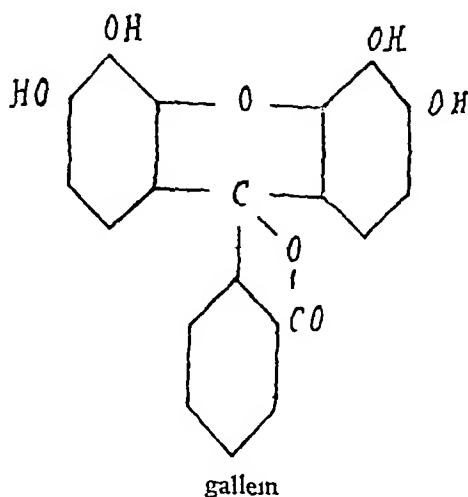
A comparatively high activity was manifested by the following —quinol (1 432000), mercurochrome-220-soluble (1 76800), catechol (1 48000), alpha-nitroso-beta-naphthol (1 25600), toluhydroquinone (1 14400), 2 4-diaminophenol (1 12800), carvacrol (1 11200), 2 4 6-trichlorophenol (1 10000)

As a class the phthalein dyestuffs have a relatively small bactericidal value, and mercurochrome owes its activity to the labile mercury in its molecule

On reduction, whereby the lactone linkage is opened up, fluorescein yields fluorescin, which is colourless



By condensing phthalic anhydride with pyrogallol *gallein* is obtained This phthalein is 4 5—dihydroxy-fluoresceine or 3 4 5 6—tetrahydroxyfluorane



It may be seen from Table VIII that —

- 1 Phenolphthalein is less powerful than phenol
- 2 Alkylation, halogenation, and esterification increase the bactericidal power of phenolphthalein

by heating a mixture of finely minced goats' flesh (1 kilo) with hydrochloric acid (150 c c) in water (2 litres) at three atmospheres pressure for a period of six hours. At the end of the process a very perfect solution of the flesh was obtained, the filtered solution being acid and, therefore, detrimental to the growth of organisms, was neutralised with caustic soda, which resulted in the formation of some 80 grammes of common salt. To bring the salt content of the medium to 0.5 to 0.75 per cent, it was made up to 12 to 16 litres with water. But the nourishing elements of the liquid were much diluted (3), the medium had a dark brown colour due to various decomposition products which were likely to inhibit microbial growth, and it was very variable in composition (4).

*C. Gibson's Modifications of Warden's Medium*—To obviate these difficulties, Gibson (5) first attempted to decolourise the medium with charcoal. This was effected by stirring the acid digest with charcoal and straining. The subsequent neutralisation threw down a copious precipitate of phosphates which further carried down the colouring matter. Though greatly improved in its appearance, the medium still remained unreliable in composition. To avoid the great dilution necessary to bring down the salt content of the medium, Gibson next reduced the quantity of hydrochloric acid to 80 c c, the proportion of the other materials and the time of heating being the same. The resulting product was then diluted with twice its volume of water. A fairly good medium was thus produced, but it still proved unreliable. Further experiments showed that in the attempts at getting as perfect a solution of the flesh as possible, the more nutritious constituents were carried beyond the stage of albumoses or peptones. In consequence Gibson not only reduced the amount of acid but heated the mixture of acid and flesh for a longer time at a lower temperature. Finely minced flesh (1 kilo) was mixed with hydrochloric acid (80 c c) and macerated for a week at 70°C, the resulting emulsion was then diluted up to three litres and heated at 3 atmospheres pressure for three hours only. In this way, the product of one kilo of flesh required only some six litres of water for dilution, and the cultivation medium was twice as concentrated as that used before. For some years Haffkine adopted this modified form of Warden's medium.

*D. Gibson's Medium or the Present Official Broth*—During the later part of 1904, Gibson carried out experiments to ascertain the effect of autoclaving subsequent to maceration of goats' flesh. The results showed that there was practically no increase in the total solids and that there was no appreciable difference qualitatively between the autoclaved medium and the other. Parallel cultivation experiments were next carried out with the autoclaved and the simply macerated flesh broths, the advantage, if any, seemed to lie with the medium which had not been autoclaved. So, Gibson did away with heating the medium under pressure of steam altogether, and gave his attention to the period of time during which it was desirable to carry on maceration at 70°C. He noted that there was a steady increase of the total solids with the length of the period of maceration, and this he subsequently found to be due to evaporation. Granting that the more nutritious constituents of the flesh are likely to be hydrolysed first and to undergo splitting-up processes by further heating, it seemed desirable to stop short of converting the entire albumen into albumose and peptone. Gibson

- 4 The tetraiodo derivative is active, but slow in acting
- 5 Complete chlorination of the phthalic-anhydride residue increases the bactericidal value
- 6 Nitration increases the bactericidal power of fluorescein and its derivatives
- 7 The high toxicity of mercurochrome to *B. pestis* is due to the labile mercury present in the molecule of dibromofluorescein

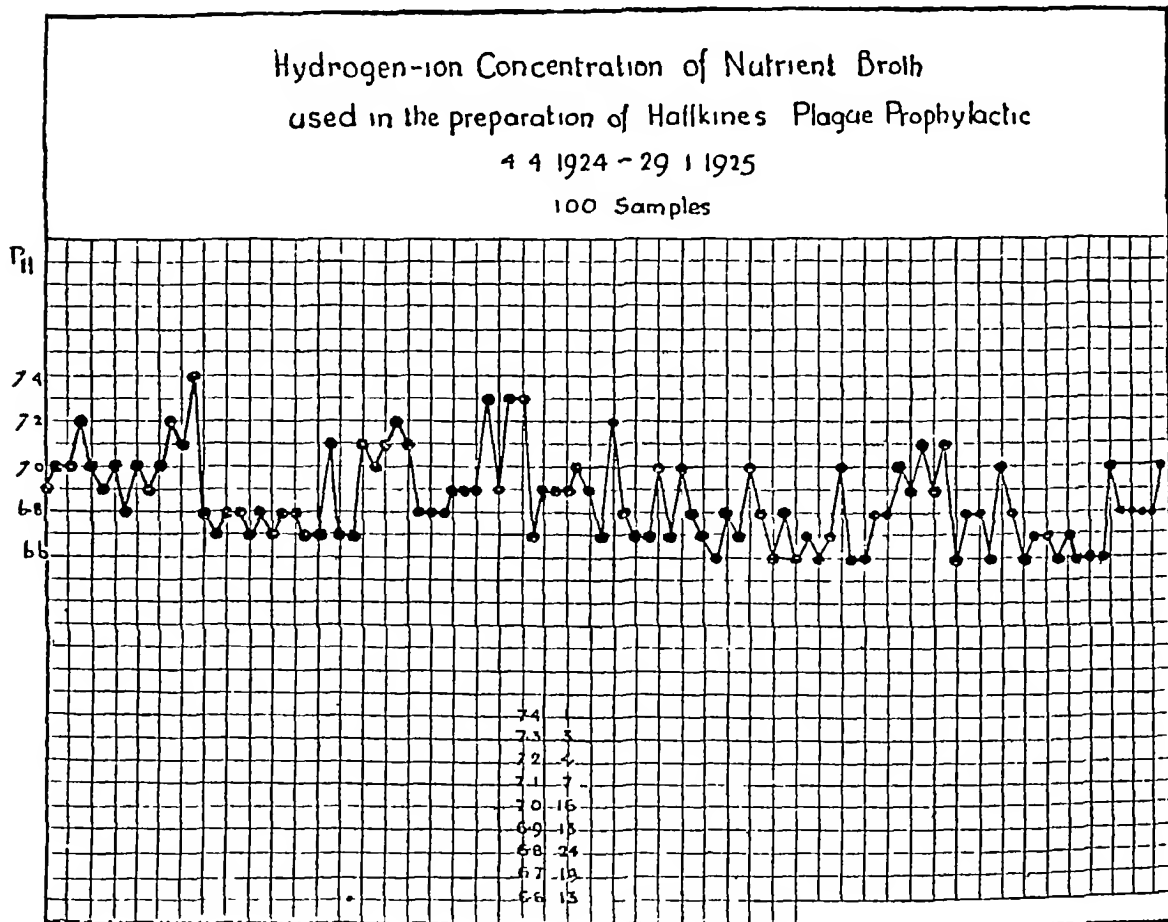
TABLE X  
*Fluorescein and related Dyestuffs*

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact	24 hours contact.
Sodium fluorescein	1 150	1 150
Potassium fluorescein	no action	no action
Fluorescein	1 600	1 800
1 8-dimethylfluorescein (orcinolphthalein)	1 600	1 800
Monobromofluorescein	1 200	1 300
4 5- dinitrofluorescein	1 300	1 600
4 5-dibromo-2 7-dinitrofluorescein (safrosin)	1 600	1 1200
2 7-dibromo-4-hydroxymercurifluorescein (mercurochrome-220)	1 3200	1 76800
2 4 5 7- tetrabromofluorescein (eosin)		
sodium eosin	no action	no action
potassium eosin	no action	no action
ammonium eosin	no action	no action
monoethyl eosin (ethyl carboxylic ester)	no action	no action
1 8-dimethyl-2 4 5 7-tetrabromofluorescein	1 600	1 800
3' 4' 5' 6'-tetrachloro-2 4 5 7-tetrabromofluorescein	no action *	1 600
4 5-di-iodofluorescein	1 100	1 800
2 4 5-tricodofluorescein	no action *	1 400
2 4 5 7- tetraiodofluorescein	no action	1 1600
3' 6'- dichloro- 2 4 5 7- tetraiodofluorescein	no action	1 100
3' 4' 5' 6'-tetrachloro-2 4 5 7-tetraiodofluorescein	1 100	1 1200

\* active in 1 100 concentration after 1-hour contact.



hundred samples of broth employed for the manufacture of the plague prophylactic between the 4th of April, 1924, and the 29th of January, 1925. These results are summarised in the form of a graph. As seen from the graph, the hydrogen-ion concentration of the culture medium varied from pH 6.6 to pH 7.4 and in 85 samples it lay between pH 6.6 and pH 7.0.

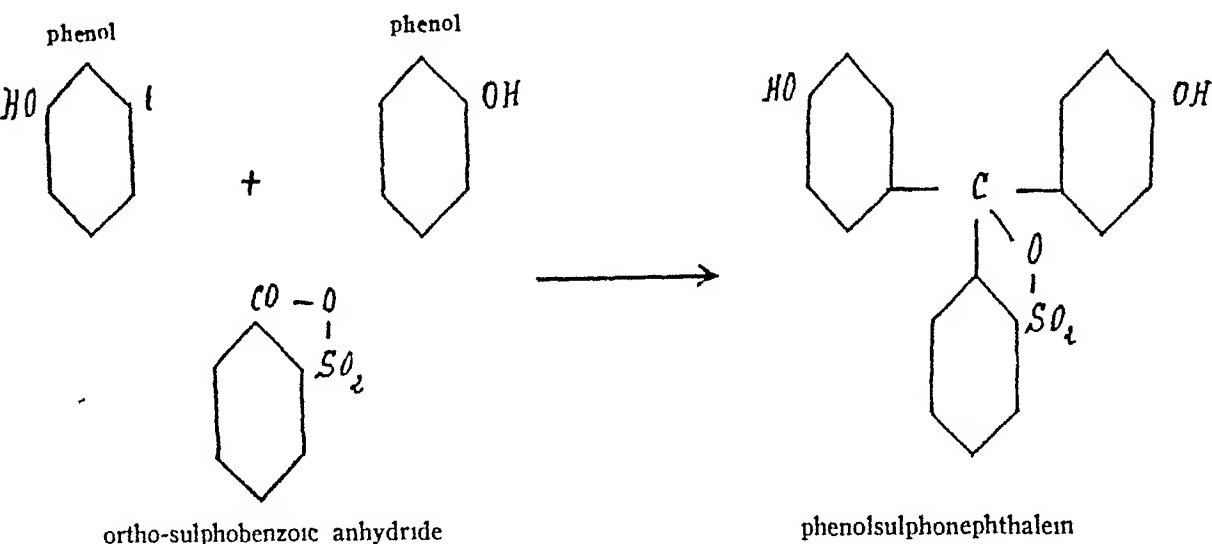


#### EFFECT OF THE INITIAL HYDROGEN-ION CONCENTRATION OF THE MEDIUM ON THE TOXICITY AND POTENCY OF THE PLAGUE PROPHYLACTIC

In view of the slight variations noticed in the hydrogen-ion concentration of the several samples of the medium examined, although their limiting values lay between pH 6.6 and pH 7.4, and in view of the fact that the prophylactic, though brewed under more or less identical conditions, occasionally yields a vaccine of very low potency as estimated on rats, we carried out an experiment to test the toxicity and potency of vaccines brewed in broth of different hydrogen-ion concentrations ranging from pH 6.0 to pH 7.2, the technique employed being identical with that employed in the manufacture of the plague prophylactic. The

## SULPHUREINS

If phenols are condensed with the anhydride of orthosulpho-benzoic acid instead of phthalic anhydride, the compounds formed are analogous with the phthaleins, and are known as sulphureins, or sulphonephthaleins



As seen from Table XII —

- 1 The sulphonephthaleins are more powerful than the corresponding phenols
- 2 Bromination lowers the bactericidal power

TABLE XII  
Sulphonephthaleins

	BACTERICIDAL CONCENTRATIONS	
	15 minutes contact	24 hours contact.
phenolsulphonephthalein	1 800 .	1 1200
tetrabromphenolsulphonephthalein	1 400	1 600
0- cresolsulphonephthalein	1 1600	1 1600
dibrom -0- cresolsulphonephthalein	1 400	1 400
thymolsulphonephthalein	1 1200	1 1600

## SUMMARY.

The bactericidal action of the commoner phenols and of some of their derivatives on *B. pestis* has been studied *in vitro* with a view to correlating their chemical constitution and bactericidal power, and, ultimately selecting or synthesizing a germicide likely to be efficacious in the treatment of plague

The method adopted was the so-called 'inhibitory method'



# A NOTE ON THE NUTRIENT BROTH NOW USED FOR THE CULTURE OF *BACILLUS PESTIS* AND ITS HYDROGEN-ION CONCENTRATION

BY

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[Received for publication, March 12, 1927]

## INTRODUCTION

HAFFKINE'S Plague Prophylactic is essentially a culture of plague bacilli grown in a liquid medium for six weeks at room temperature, and sterilised by heat at 60°C for 15 minutes, to ensure further its sterility, it is carbolised so as to contain 0.5 per cent of phenol

## THE NUTRIENT BROTH

*A The Standard Broth*—When the prophylactic was introduced as a preventive measure against plague, Haffkine(1) grew the plague bacillus in broth containing 1 per cent commercial peptone, the one ordinarily employed in European laboratories for the cultivation of organisms

*B Warden's Medium*—In consideration of the prejudice of the people of India against the use of commercial peptone(2) and the difficulty in obtaining a proper supply of that product, which of necessity had to be bought in Europe, the manufacture of peptone from goats' flesh was undertaken in this laboratory by Warden, and the new product came into use towards the end of 1898

Warden's method of preparing the culture medium is based on the long known fact that albuminous matter can be hydrolysed and rendered incoagulable by heat through the prolonged action of dilute acids. The medium was prepared

spores are thinner and more delicate than those of *Sarcocystis miesheriana* or of any other variety described. Also both their ends are somewhat pointed and not blunt at one end like those of *Sarcocystis miesheriana*. No terminal filaments could be seen. In stained specimens (Leishman's stains very well) the nuclei are well seen and are placed either in the centre or more commonly subterminally. The nucleus is oval and is placed along the long axis of the spore. It is  $3.33\ \mu$  long and  $1.66\ \mu$  broad (i.e., as broad as the spore itself). In some spores (well seen in specimens stained with Leishman and not seen in unstained specimens) one end is stained with a deep purple nuclear stain which fades off imperceptibly towards the centre. This stained area at the end is somewhat shorter than the nucleus and is as broad as the spore. These are seen only in some spores. Are these the 'Polar capsule' of Reill? Is it possible that these represent spores in certain stage of development? In the protoplasm of all the spores are seen dark (almost inky) very fine granules two or three in number, usually at one end of the nucleus. The spores appear to have a definite membrane or cuticle. No vacuoles could be seen in either stained or unstained specimens.

The question arises. Is the ulceration due to the *Sarcocystis* (it is stated that a powerful toxin called Sarcocystin is elaborated by this parasite) or is it merely a chronic ulceration over a muscle infected with this parasite? Sections of the ulcer, however, do not show any cysts or spores either in the skin or in the subcutaneous tissue in the ulcerated area and they are only present in the otherwise normal fibres. It is suggested that this parasite is an undescribed species of sarco-sporidium infecting the human host for the following reasons—

- 1 The naked eye appearances are peculiar. The cysts of this parasite are much thinner and longer than those of *Sarcocystis miesheriana* and are just visible to the naked eye.

- 2 The spores are smaller than those of *Sarcocystis miesheriana* and besides, both their ends are pointed instead of being blunt at one end as in *Sarcocystis miesheriana*.

- 3 In *Sarcocystis miesheriana* the nuclei are stated to be always terminal but in the present case they are not always so, as in a number of them the nuclei are also centrally placed.

- 4 Absence of any vacuoles in the spores.

The special interest in this case is. Firstly, the fact that while sarcocystic (*Sarcosporidial*) infection is common in cattle and other animals it is very rare in man (only about six cases seem to be recorded) and secondly, that it has special features not described in other varieties of this parasite.

My thanks are due to Captain P. N. Basu, I.M.S., Professor of Pathology, Medical College, Madras, for kindly permitting me to publish this article.

found that in the broth macerated for three days, about 75 per cent of the presumably available albumen is converted into albumose and peptone. Parallel cultivation experiments were carried out with macerations of three and seven days, respectively. On examination of the comparative cultures after two and four days, it was found that macerations of three and seven days were giving better growths than the autoclaved material. Further, it appeared that three days maceration was better than seven days. Thus an improvement in the manufacture of broth was effected (1905) and resulted both in economy and efficiency. Bannerman(6) employed this modified medium for the manufacture of the plague prophylactic in 1905, and it has been in use ever since.

The preparation of this medium is as follows—Goats' flesh freed of fat and gristle is finely minced in a mincing machine, weighed, and placed in a jar. To every kilogramme of flesh 80 c.c. of hydrochloric acid are added and the contents of the jar are thoroughly mixed, the jar is then placed in a hot-water-tub and kept at a temperature of 70°C for three days, on the fourth day the jar is taken out and two litres of boiling water added for every kilo. of fluid, then from 60 to 80 c.c. of a 40 per cent caustic soda solution are added sufficient to make the fluid slightly alkaline to litmus paper. The diluted peptone is poured into a jar and placed in the steam steriliser for one hour at 100°C (at ordinary atmospheric pressure). The jar is then taken out of the steriliser, and the contents filtered through filter paper. The filtrate is measured and to every litre two litres of boiling water are added. One litre of this dilute peptone solution is filtered into each of a number of flasks through filter paper, the flasks are plugged with cotton-wool and placed in a steam steriliser for one hour using saturated steam at 15 pounds above the atmospheric pressure. The medium is now ready for inoculation with plague.

#### THE HYDROGEN-ION CONCENTRATION OF THE CULTURE MEDIUM

Haffkine(7) thought that an acid medium is inimical to the growth of the plague germ. Stewart(8) carried out experiments in this laboratory on the effect of different degrees of alkalinity upon the growth of the plague bacillus. Mayr(9) found that a very lightly alkaline reaction of the medium favoured the growth of *B. pestis* whilst in a slightly acid medium, growth began to show itself only after two or three weeks of incubation. Bannerman(10) found that broth slightly acid to phenolphthalein is more favourable to the growth of the plague bacilli than an alkaline one, that a highly acid broth is inimical to the growth of plague bacilli, and that the plague germ soon dies out in such a medium. Within recent years, our knowledge of the adjustment of laboratory media has been extended by the introduction of the newer physico-chemical conception of hydrogen-ion concentration. It has now been shown that various bacteria have definite optimum hydrogen-ion concentrations at which their functional activities are at a height, and limiting hydrogen-ion concentrations beyond which their life cycles are arrested.

With the aid of a colorimeter and using 0.01 per cent solution of phenol-red as our indicator, we have determined the hydrogen-ion concentration of one



Fig 1

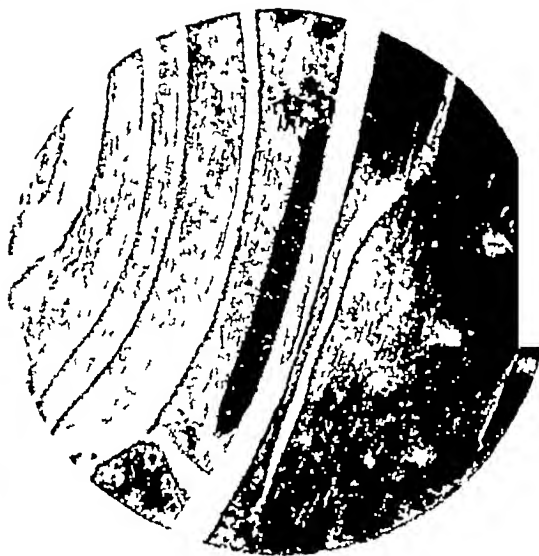


Fig 2

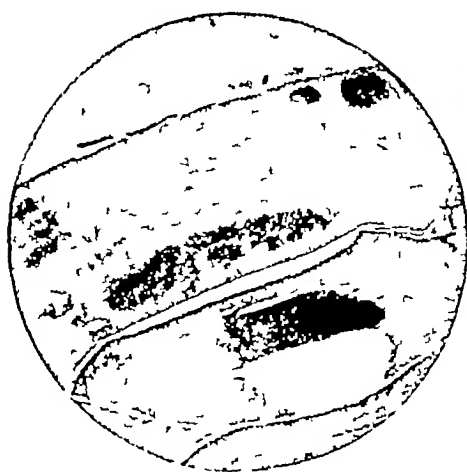


Fig 3



Fig 4

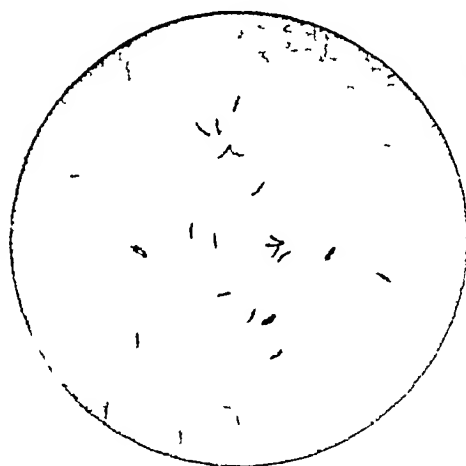


Fig 5

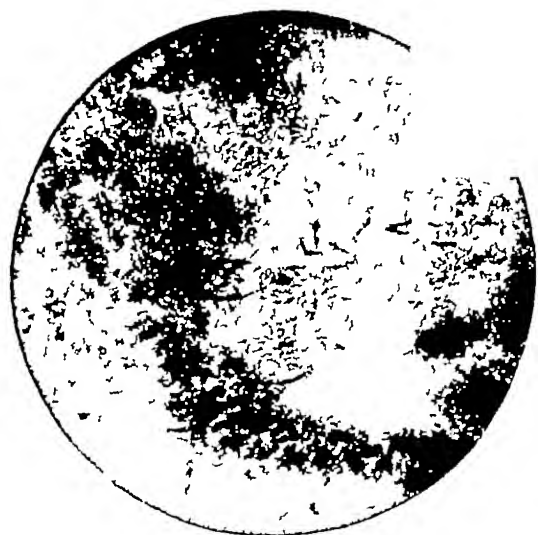


Fig 6.

results are summarised in the following table, and show that the highest percentage immunity was obtained with a broth of the initial pH 6.8 —

TABLE I

Vaccine Material	Incubated for weeks	Original pH of the broth used	No of Rats used	Per cent 'Toxic' deaths	Survivors	Total deaths	Percentage Immunity
Vaccine brewed in official broth	6	6.0	25	8.0	23	17	26.0
	6	6.2	25	4.0	24	20	16.6
	6	6.4	25	20.0	20	11	45.0
	6	6.6	25	20.0	20	13	35.0
	6	6.8	25	16.0	21	5	76.1
	6	7.0	25	8.0	23	18	21.7
	6	7.2	25	12.0	22	17	22.7
Controls					10	10	0.0

## SUMMARY.

1 Successive improvements in the constitution of the broth used for Haffkine's Plague Prophylactic are described

2 The hydrogen-ion concentration of the broth has been estimated in samples from one hundred different brews

The pH values lay between 6.6 and 7.4 and in 85 cases between 6.6 and 7.0

3 In a small experiment involving 175 rats the most favourable immunity rate was shown from vaccine made from broth of which the initial hydrogen-ion concentration was 6.8

4 The existing method of adjusting the reaction of the culture medium in this laboratory employed in the manufacture of the prophylactic, satisfies the requirements of the hydrogen-ion concentration for the optimum growth of the plague bacilli

## REFERENCES

- (1) *Ind Med Gaz*, June 1897 Preparation of the prophylactic by Haffkine
- (2) *Proc Roy Soc of Edinburgh*, Vol 24, Part 2, p 117
- (3) Summarised Report on the Bombay Plague Research Laboratory for 1896—1902, p 12
- (4), (5) and (6) Report of the Plague Research Laboratory for the official year ending 31st March 1905, pp 3, 5, 14 and 15
- (7) *Sci Mem by the Officers of the Med and San Depts of the Govt of India*, new series, No 33, p 1
- (8) Summarised Report on the Bombay Plague Research Laboratory for 1896—1902, p 11
- (9) Report of the Indian Plague Commission, Vol 3, pp 18—21
- (10) *Sci Mem by Officers of the Med and San Depts of the Govt of India*, new series, No 33, pp 11-12



EXPLANATION OF PLATE XXIV

- Fig 7 Naked eye appearance of a muscle of man infected with sarcosporidium  
The cysts (Miescher's tubes) are just visible to the naked eye as thin  
long white hair-like streaks
- „ 8 Surface view of the ulcer of chest
- „ 9 Spores of sarcosporidium magnified (eye-piece No 3 and objective  
1|12" oil immersion) Specimen stained with central and subterminal  
nuclei and dark granules in the protoplasm and the dense staining of  
the protoplasm at one end

# A CASE OF SARCOSPORIDIAL INFECTION IN MAN

BY

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[Received for publication, March 14, 1927]

CLINICALLY, the lesion consisted of an ulcer over the chest wall around the nipple, with much inflammation of the subcutaneous tissue. It was excised as a carbuncle and sent for pathological examination. No clinical history of the case could be obtained. The specimen shows an irregularly oval ulcer about  $2\frac{1}{4}'' \times 1\frac{1}{2}''$ . The margins of the ulcer are serpigenous, edges somewhat thickened and raised and there is a bluish zone all round it. The base is undermined, and shows medium sized reddish brown granulations. The ulcer has the appearance of a chronic ulcer. On cutting into it, it is seen that the ulceration involves the skin and subcutaneous tissue only and not the muscle underneath which is somewhat paler than normal. On carefully examining the muscle a number of very thin, long, hairy, whitish streaks are seen in the muscle fibres and running parallel to them. They are just visible to the naked eye. They are seen throughout the entire length of the muscle fibres in the specimen. On cross section, they are seen as tiny white pin points just visible to the naked eye. These are the sarcosporidial cysts—the so-called 'Miescher's tubes'.

On dissecting these 'tubes' they are seen as thin long cystic bodies with a definite capsule and packed full of large numbers of granular bodies—the spores. The length of these cysts is somewhat over 5.3 cm as the longest specimen dissected was incomplete at either end and occupied the entire length of a muscle bundle in the specimen. The breadth (including the sheath or capsule) is 0.322 mm. The sheath proper is clear and homogeneous and is 0.0161 mm (or  $16.1 \mu$ ) thick. External to this is a false sheath formed by compressed muscle fibres showing clearly their nuclei and striation. From the true capsule, numerous septa run into the cyst dividing it into a number of partitions giving it a honeycombed appearance. These septa are well seen in the centre of the cyst. On crushing one of these cysts numerous tiny curved bodies are liberated. These spores are somewhat crescentic and are  $8.33 \mu$  long and  $1.66 \mu$  broad, and look very much like the crescents of malignant tertian malarial parasite. These





marked off by a mountain range commonly referred to as the Sahyadris or Western Ghats. Nearly the whole of the Gujerat Division of Bombay is a low-lying plain, in few places rising more than a few hundred feet above sea level. The Konkan Division of Bombay is the coastal strip extending from Gujerat to the southern extremity of the Presidency, and rising, usually within a distance of 50 miles, to the crest of the Western Ghats at an elevation of between 3,000 and 5,000 feet.

The western portion of this part of India is supplied with rain by the Arabian Sea or South-west monsoon, and the eastern portion by the Bengal or South-east monsoon. The Arabian Sea current, striking the sharp rise of the Western Ghats, delivers a very heavy rainfall on their western slopes, in most places ranging from 100 to 150 inches a year. As soon as the crest is passed the annual rainfall drops with striking suddenness to between 20 and 30 inches, leaving a broad strip of the western Deccan, 100 to 200 miles in width, with a very arid climate. North of Bombay the rainfall rapidly diminishes, falling to about 40 inches at Surat, 35 at Baroda, 20 to 30 in Kathiawar and 12 to 20 in Cutch. The Bengal monsoon, although carrying the bulk of its rain into Bengal, Assam and Burma, passes north and a little north-west from near the mouth of the Godavari River and supplies a moderate rainfall to a broad belt of country including Chota Nagpur, most of the Central Provinces, and part of the Central India Agency. Western Hyderabad, Berar and the western parts of Central India are in a precarious position between the two monsoon currents, this results in these areas having a very variable annual rainfall, moderately heavy in some years and very light in others. For the most part the rainfall is sufficient in normal years for the principal crops, wheat and cotton, and therefore artificial irrigation is by no means as common as in the drier parts of north-west India.

#### HUMAN INFECTION

The only information hitherto available concerning helminthic infections in Central India and Bombay has come from a few isolated hospital records and a few examinations made in jails and asylums in the Central Provinces and in the Grant Medical College in Bombay. Banatvala found 25 per cent of 251 prisoners in the Central Jail at Nagpur infected, and 31 per cent of 266 inmates of a Nagpur Asylum. Souza found 41 per cent and 34 per cent infected in two lots of prisoners of 571 and 850 individuals respectively in the Central Jail at Raipur. Reancy reported 66 per cent of 69 individuals in Wardha District infected, and 14 per cent of 97 jail inmates. These jail figures, however, are of very little significance so far as their own districts are concerned, since they include individuals from all parts of the Province. A scurvy-like disease in the Akola Jail was attributed to the presence of hookworm infections. In Bombay hookworms were found in only 7 of 254 post-mortem examinations, and in 7 of 235 examined at the Grant Medical College. These are the only published records found by the International Health Board (1922) in a search of the literature on the subject. Lt-Col Stiles

### EXPLANATION OF PLATE XXIII

- Fig 1 Portion of a cyst (Miescher's tube) showing the capsule Low power  
„ 2 Section of an infected muscle showing the cyst cut longitudinally (stained with Hæmalum and Eosin)  
„ 3 Section of an infected muscle with a cyst cut lengthwise showing the spores densely packed in the periphery and empty in the middle (stained with Hæmalum and Eosin)  
„ 4 Cross section of a cyst more highly magnified showing the cyst wall and the septa in the middle where the spores are absent (stained with Hæmalum and Eosin)  
„ 5 The spores having the typical crescentic shape under high power (1|12" oil immersion)  
„ 6 The spores more highly magnified showing the nuclei (1|12" oil immersion)
-





Fig 7

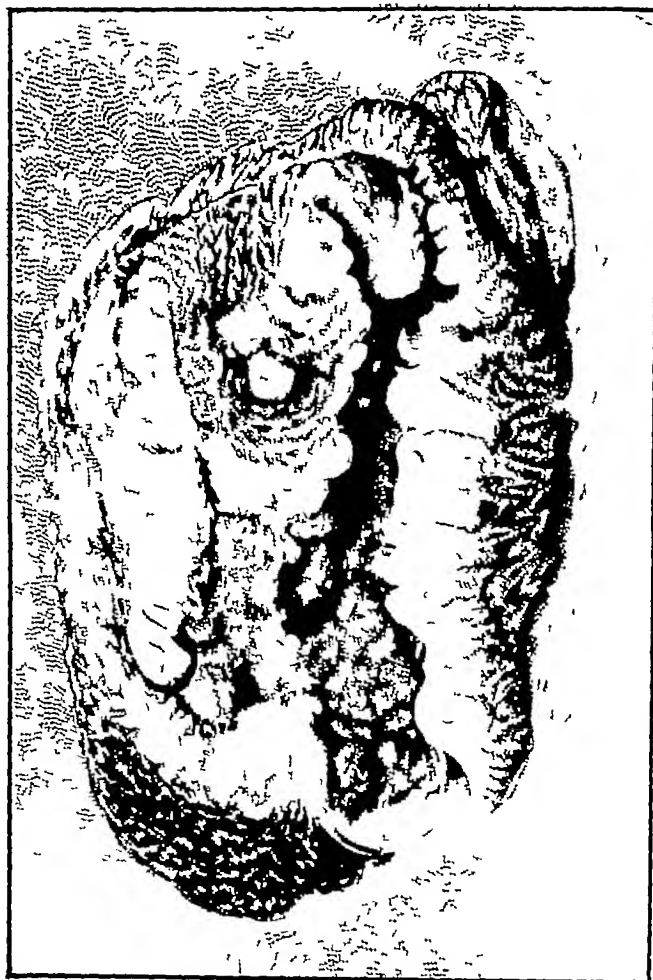
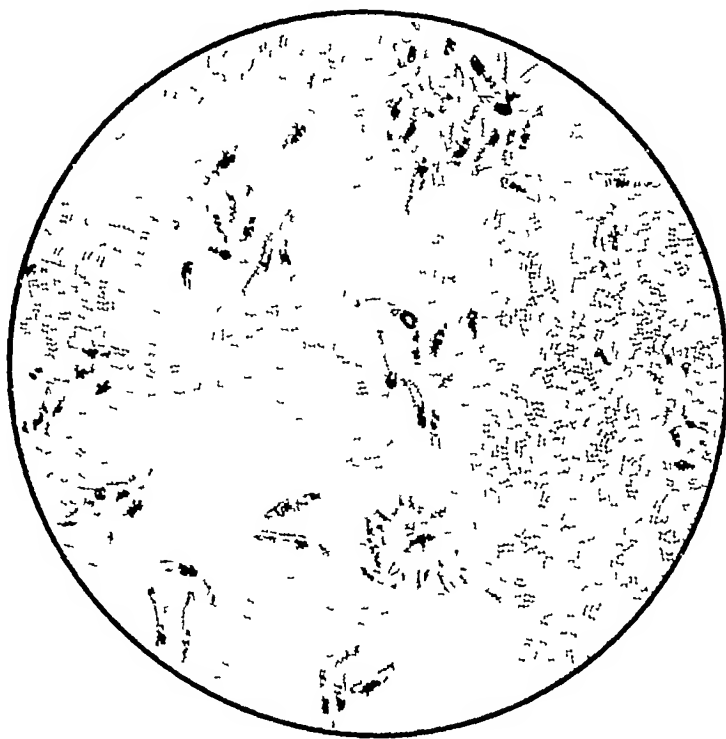


Fig 8





the Bay of Bengal, but one or both sources of rain may fail, hence the precipitation in this region is very variable in different years, and is largely limited to a period of two months. In the Bundelkhand area the annual fluctuations are still greater, and the occurrence of a good rainfall still more uncertain. The area lying at the head of the two opposite monsoon currents is represented in our survey by Bhopal and Bina, the Bundelkhand area in Central India was not investigated, but Jhansi and Banda in the portion of this area lying in the United Provinces have been studied and previously reported on. In the narrow strip above mentioned, from the western border of the Central India Agency to the Bundelkhand, the index of infection as indicated by Bhopal and Bina is just a little under 50, which is extremely light. Judging from conditions found at Jhansi and Banda it is still lighter in the Bundelkhand. Judging by the results obtained at Ujjain and at Udaipur and Kotah in Rajputana, just north of Central India, the whole of the northern portion of Central India and Gwalior is practically free from hookworm infection.

The eastern half of the area here considered, including nearly the whole of the Central Provinces and the eastern two-thirds of Hyderabad, is supplied with rain by the Bengal monsoon striking in from the coast in a north-westerly direction, the amount of rain gradually decreasing from south-east to north-west. This area is represented by investigations at Bilaspur, Jubbulpur, Nagpur, Nander, and Hyderabad. Of these places Bilaspur, lying in a rice-growing plain with favourable soil, had the highest degree of infection, the index being 177. Jubbulpur, with a lower rainfall in a more elevated wheat-growing country had an index of 102, whereas the village studied near Nagpur, in an area of comparatively low rainfall and black cotton soil, had practically no infection. It is probable, however, that in areas in the vicinity of Nagpur and in eastern Berar where local conditions of soil and drainage are more favourable for the propagation of hookworm, a low degree of infection, comparable with that at Bhopal and Nander, exists. The index of infection at Nander in northern Hyderabad was 42 whereas in two villages near Hyderabad the indices were 70 and 68. Probably nowhere in Hyderabad does the index exceed 75, but it gradually decreases from that degree of infection in the south-east to practically complete absence in the west and north-west.

To sum up, there is no appreciable amount of hookworm infection in any parts of this great area except in the Konkan Division of Bombay and in a small area in the eastern part of the Central Provinces, largely limited to the Districts of Raipur, Bilaspur, and part of Drug. Even in the parts of these districts which have black cotton soil, the degree of infection is probably very low. Our map showing the probable extent of areas having different degrees of hookworm infection gives a rough idea of the distribution. It is probably not far from correct in its general configuration, although the delimitation of the different grades of infection is likely to vary somewhat with the type of soil, black cotton soil is apparently unfavourable for hookworm propagation, and requires a much heavier rainfall to keep it moist.

# THE PREVALENCE AND EPIDEMIOLOGY OF HOOKWORM AND OTHER HELMINTHIC INFECTIONS IN INDIA

## Part X.

CENTRAL INDIA AND BOMBAY (CENTRAL PROVINCES,  
CENTRAL INDIA AGENCY AND GWALIOR, BOMBAY PRESIDENCY  
EXCEPT SIND AND HYDERABAD)

BY

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THE portion of India included in this paper consists of the Central portion of the Indian Peninsula and the west coast from Sind to the borders of the Madras Presidency. The total area comprises over 440,000 square miles, with a population in 1921 of 63,239,321. This is about  $1\frac{1}{2}$  times the population of Bengal, although the area is well over five times as large. The most densely populated sub-division is the Gujerat Division of the Bombay Presidency with 292 persons per square mile. Next comes the Konkan Division of Bombay with 222 per square mile. Throughout the upland or Deccan Divisions of the area the population density lies for the most part between 100 and 150. The area of lowest density is the Chota Nagpur Division of the Central Provinces, which has only 61 per square mile. The distribution of the area and population in the different divisions is as follows —

Division	Area	Population	Density
Central Provinces	131,377	15,979,660	122
Central India Agency	51,531	5,997,028	116
Gwalior State	26,357	3,186,075	121
Hyderabad State	82,698	12,471,770	151
Bombay excluding Sind	148,695	25,604,793	172

The greater part of the entire area lies on the great Central Indian Plateau known as the Deccan. On the west this region is fairly sharply

northern Gujerat, and in a few places in the western Deccan (e.g., Jalgaon) the people habitually go bare-footed in and about their villages. In the places mentioned the majority of the adult males wear shoes, but this is of no importance since in these places hookworm infections could not develop anyway on account of climatic conditions. As in other parts of India the people prefer to stand on dry ground during defæcation, therefore the presence in some places of extensive irrigation has no practical effect so far as hookworm is concerned. In some parts of the Deccan, e.g., Poona, the possibility of infection resulting from ground kept moist by irrigation is further reduced by a law forbidding the irrigation of ground nearer than one-fourth of a mile from a village, as an anti-malarial measure. It would, therefore, never be possible for conditions to develop such as those found in villages in the N W Frontier Province, where the irrigated land comes right up to the walls of the village, making a certain amount of defæcation on wet ground inevitable.

In the southern portion of the Bombay coast the village conditions become more like those on the Malabar coast of Madras. The houses are not built in compact groups but are scattered, with large individual compounds and usually with more or less jungle intervening between them. The result of this is that each household, for the most part, has its own defæcation areas, and there is little opportunity for intermingling of large numbers of villagers in common defæcation areas. Our experience has invariably been that this tends to lower the amount of infection in a community as a whole, and to make it more irregular. Among people living under practically identical conditions some will be found with extremely light infections while their neighbours may have very heavy ones. The heavy rainfall and relatively long rainy season on the south-western coast of India, together with favourable soil conditions, abundance of shade, absence of excessive temperatures, and individual habits ideal for hookworm propagation, would lead to a much heavier infection than the moderately heavy one which actually exists, if the people lived in compact villages as in most other parts of India. It is the same factor which has a certain amount of protective influence in parts of eastern Bengal, Assam and Burma. The abundance of dense jungle and forest, affording unlimited suitable places for defæcation, is also a factor in keeping down the degree of infection, since the stools, even of individual households, are often widely scattered, thus lowering the probability of standing on infested ground during the act of defæcation.

The nature of the soil unquestionably has an important influence on the degree of infection. This is especially in evidence in the Central Provinces and Central India where there are large areas of black cotton soil, a soil which is particularly unfavourable for the development and survival of hookworm larvæ. It requires over twice as much water to make it wet as is required for ordinary alluvial soil, it consists of very fine particles through which hookworm larvæ cannot easily migrate, and it tends to dry into extremely hard clay-like clods when exposed to the sun and dry atmosphere of the parts of the country where it is found. I think it is very doubtful



rainfall In flat country such as most of the Bilaspur District a much lighter fall of rain is sufficient to keep the ground moist than is the case in undulating or hilly country where much of the water runs off instead of soaking into the ground

The temperatures existing in much of the western part of the country here considered, with the considerable day and night variations which exist on the tableland, even in the monsoon season, are too high to be entirely favourable In this respect the Bombay coast is much more favourably situated Tables II and III show the essential facts concerning rainfall and temperature in the different parts of the area under consideration

TABLE II

*Mean Maximum and Minimum Temperatures in Various Localities in Central India and Bombay*

Locality	COLDEST MONTHS		HOTTEST MONTHS		MONSOON SEASON	
	Mean max	Mean min	Mean max	Mean min	Mean max	Mean min
Nagpur	81—83	54—56	105—109	80—82	87—88	74—76
Indore	79—80	49—50	100—103	76—77	83—85	70—73
Hyderabad	81—85	57—59	101—103	78—79	88—90	72—74
Rajkot	83—85	51—53	102—105	75—78	89—91	74—76
Bombay	82—83	68—70	89—91	80—81	84—85	77—78
Ratnagiri	86—87	66—67	90—91	78—80	84—85	75—76
Poona	85—86	53—55	100—101	71—73	82—83	69—71
Sholapur	86—87	58—59	103—105	75—77	88—89	71—72

Since the investigations in Central India and Bombay were made for the most part in November, about six weeks after the close of the wet season, the infections found can be considered as near the maximum, and no correction is necessary to allow for worms lost during a period when re-infection ceases

#### OTHER HELMINTHIC INFECTIONS

##### *Ascaris and Trichuris*

*Ascaris* and *Trichuris* infections are comparatively uncommon throughout Central India and Bombay except on the Bombay coast With the exception of the latter area and in the village of Bholakpur near Hyderabad city, the incidence of *Ascaris* infections nowhere reaches 10 per cent Except at Bhayndar near Bombay, *Trichuris* infections were everywhere rare and in most places entirely absent, as will be seen by reference to Table I An incidence of 3.3 per cent was found at Bholakpur, which had 22 per cent of *Ascaris* infections, 1 per cent at Dharwar, which had 82 per cent *Ascaris*

Webb, I M S, Director of Public Health of the Central Provinces, kindly sent me a report on hookworm examinations in the Central Jail at Jubbulpore. Of 696 examinations in 1925, 16.5 per cent were found infected, and of 1,145 in 1926, 16.8 per cent. Of these only 212 and 592 respectively, with 17.9 per cent and 8.1 per cent infections, were from Jubbulpore District. In general higher incidences of infection were found in prisoners from the eastern and northern parts of the Provinces than from elsewhere.

Our own investigations were made at villages near Bilaspur, Jubbulpore, Bina and Nagpur in the Central Provinces, near Bhopal and Ujjain in Central India, near Baroda, Viramgram and Rajkot in the Gujarat Division of Bombay, near Bombay in the Konkan Division, and near Sholapur, Dharwar, Poona, Ahmednagar, Jalgaon, Nander and Hyderabad in the Deccan. The detailed results of the examinations made in these places are shown in Table I.

As might be expected from the great variation in climatic conditions in different parts of this area, there are considerable differences in the amount of hookworm infection present. The western part of the Deccan as represented by Poona, Sholapur, Ahmednagar and Jalgaon is practically entirely free from the infection throughout a strip probably about 200 miles in width. Likewise Kathiawar and northern Gujarat, represented by Rajkot and Viramgram, lying north of the Arabian Sea monsoon current, are also practically free. Unfortunately the village selected for study on the Bombay coast cannot be considered truly representative, since it is situated on marshy ground directly on the coast. The incidence of infection here was only 54.2 per cent with an index of infection of only 50, whereas near Baroda in the path of the monsoon current as it swings inland from the Bay of Cambay, 78.3 per cent were found infected, and the index of infection was 96. Baroda has a much lower rainfall than the vicinity of Bombay, and the conditions favouring hookworm infection are no better. There appears to be little doubt but that in typical villages in the vicinity of Bombay City the index of infection will be found to be not only much higher than that found at Bhayndar but also considerably higher than that at Baroda. I think it likely that the index of infection will be found to be between 150 and 200 in this locality, increasing with the longer rainy season as one proceeds further down the coast. At Mangalore in the South Kanara District of Madras, about 80 miles south of the southern border of the North Kanara District of Bombay, infection is practically universal and the degree of infection is fairly high, the index being 200. It is probable that a similar degree of infection extends all along the narrow coastal strip of the Konkan at least as far as Ratnagiri.

A moderate amount of rain is carried in a north-easterly direction from the Gulf of Cambay to Central India, but the annual rainfall is below 40 inches except in a comparatively narrow strip near the middle of the Central India Agency involving Indore and Bhopal. From Bhopal to the north-western parts of the Central Provinces the rain carried over by the Arabian Sea current is supplemented more or less by rain carried north-westerly from

water for the city. This lies just across a road from the village but is fenced off and is protected from pollution by a guard. We could find no evidence of pollution anywhere around the edges of this reservoir, and believe that at present it does not serve as a source of infection. There were also several wells, but all of these were so constructed as to be proof against gross pollution. Eventually, however, there was discovered a large, shallow, cement-lined tank near the village, built to receive water from a pipe connected with the city water supply as a source of water in particularly dry years. The dry bed of this tank was frequently resorted to as a convenient place for defæcation. During the rainy season, however, the tank would fill up with rain water, and would then in all probability be used as a source of drinking water, in fact, it was admitted by some of the people that the lower castes sometimes took water from here when it was full of rain-water. This would, of course, be an ideal source of both *Ascaris* and *Trichuris* infection, yet the fact is that while 82 per cent were infected with *Ascaris*, only 1 per cent had *Trichuris*. One possible explanation suggests itself. It is only within the last three or four years that the reservoir has been utilized as a source of water for the city. Previous to that time it is quite possible that the lake, or the widening in the stream out of which it was made, was grossly polluted around its edges and at the same time served as a source of drinking water. This would inevitably have resulted in a high incidence of *Ascaris* and *Trichuris* infections. If *Ascaris* lives longer in the human intestine than *Trichuris*, it is possible that the absence of *Trichuris* is due to their having been lost in the meantime while the *Ascaris* acquired several years before had not yet been lost. This is a purely theoretical hypothesis, and one which can at present only serve as a basis for investigations to determine whether there is any truth in it. The situation at this village near Dharwar is a particularly interesting one, and a careful study of the conditions and habits at different seasons of the year and of changes which may or may not occur in the next few years, might give some valuable clues to the epidemiology of *Ascaris* and *Trichuris* infections.

Although no investigations were made in the southern Konkan, the similarity of conditions there with those obtaining in the South Kanara District of Madras leaves little room for doubt but that the worm infections in the two places will be found to be about the same. Along the Malabar coast *Ascaris* and *Trichuris* infections are almost universal. For the most part well water is used, but a great many of the wells, of which there is one for almost every household, are not protected by raised sides and may be subject to gross pollution at times of heavy rains. It is also possible when the heavy rains characteristic of this part of the country come, that other sources of water are used. Our investigations in all parts of India have shown that all localities which have very heavy rainfalls in excess of 80 to 100 inches or more a year, are severely and almost universally affected by *Ascaris* and *Trichuris* infections, whereas very dry localities with a light rainfall are almost always practically free from them. Whether this effect of a heavy rainfall is due to washing faecal material into the usual sources of drinking water

enough to allow hookworm larvæ to develop than does alluvial or laterite soil. In eastern Berar and in the Nagpur and Wardha Districts of the Central Provinces, for instance, in regions of black cotton soil, which is the predominant type of soil in this part of the country, and was the soil found at the village near Nagpur selected as typical of this area, the index of infection is extremely low, but it is probable that it is a little greater in areas having other types of soil. It is not likely, however, that the index of infection would exceed 50 in any locality in this part of the country, and such a degree of infection is entirely negligible from a practical standpoint. It is furthermore probable that the degree of infection would be appreciably greater in some years than in others, but, as was pointed out in the report on the United Provinces, the amount of infection in areas in which only occasional years are favourable does not make a cumulative acquisition of worms possible, and therefore a degree of infection sufficient to be of any practical importance can never develop.

#### DEFÆCATION HABITS AND OTHER FACTORS INFLUENCING THE DEGREE OF INFECTION

Throughout practically the whole area here considered, except the southern part of the Konkan Division of Bombay, the habits of the people are such as would lead to very heavy infections if climatic conditions were suitable. Throughout this area, with the exception noted, the villages are built in rather compact groups of mud houses, closely situated with narrow passages between, and with very small individual compounds if any are present at all. Except for an occasional public latrine in a few villages which are not sufficiently used to make any impression on the hookworm propagation, and a few private latrines in the compounds of Muhammedans in Hyderabad, soil pollution is universal. The consequence is that convenient places all around the edges of the villages are badly polluted, as a rule there are no separate defæcation areas for any particular households or for either sex. The females and children usually go to thickets or banks of tanks or streams very close to their houses, whereas the males go a little farther afield. There are certain favoured types of places which are particularly selected, such as cleared spaces in thickets of prickly pear, beds of dry streams, ditches, and the banks of tanks or rivers. In consequence, due to the use of these selected places by people from all the houses which are conveniently situated with respect to them, these places are very frequently used and become badly polluted. In so far as climatic conditions would allow the development and survival of the larvæ, these places become hot-beds of infection, and it is only the fact that in the greater part of the area under consideration the ground is seldom moist long enough to allow the larvæ to survive up to the development of the infective stage that protects the people from a very severe hookworm infection. Even in southern Gujerat and in the eastern part of the Central Provinces the rainfall is sufficient to allow a ground infestation to develop only for two or at most three months in the year. Except in Kathiawar,





if any area with this type of soil will be found to have any appreciable amount of hookworm infection. Red sandy and gravelly soils or soils largely made up of disintegrated basalt rock are common in many parts of Central India. With a sufficient rainfall to keep them moist, such soils appear to be very favourable for hookworms.

#### CLIMATIC CONDITIONS IN RELATION TO HOOKWORM INFECTION

Much has already been said about the climatic conditions especially with respect to rainfall, in the area under consideration. There is no question but that, as in north-west India, the amount and distribution of the rainfall is by far the most important factor influencing the amount of hookworm infection. It is only where the Arabian Sea current delivers a heavy rainfall on the Bombay coast, and where the Bengal current brings in a moderate one to the south-eastern part of the Central Provinces, that any appreciable amount of hookworm infection is present. The total amount of the annual rainfall is not, however, the only factor to be considered, its distribution throughout the year is also of great importance. A locality having 30 to 40 inches of rain a year in which that rain is so distributed that not more than 7 or 8 inches falls in any one month will never have a sufficient amount of hookworm infection to be worth consideration, whereas a locality with a rainfall no greater but where the majority of it falls within two months, each with 12 to 15 inches, may have several times as much hookworm infection. It is also to be noted that the number of rainy days on which the monthly rainfall is precipitated has a marked effect. A comparison of the amount of hookworm in Sholapur and Dharwar is of interest from this standpoint. During the months of June to September inclusive the average rainfall in Sholapur is 13.41 inches and in Dharwar 15.67, but in the former place there are only 21.2 rainy days in the three months while in Dharwar there are 36.4. At Sholapur only 1 extremely light hookworm infection was found in 100 examinations, whereas at Dharwar 85 of 100 had light infections. Evidently, therefore, even so little rain as 5 inches a month may be sufficient to permit the development of hookworm larvæ if distributed over about 12 days per month, whereas a similar rainfall distributed over only 7 days in a month is inadequate. In the former case the rainfall is never sufficient to keep the ground continuously moist for a long enough period to allow any appreciable soil infestation to develop, whereas in the latter case enough soil infestation can develop so that infections may ordinarily be acquired for parts of at least two or three months in the year. Furthermore, the occurrence of occasional years with a very deficient rainfall followed by years with an unusually heavy rainfall tends to produce lower hookworm infections than the occurrence of an even rainfall year after year with several months each year in which the fall is sufficient to keep the ground continuously moist. In most of Central India the rainfall, for reasons already stated, tends to be very irregular, with relatively enormous fluctuations, and there are often rainless periods of considerable length even in the middle of the monsoon season. If it were not for these facts the 30 to 45 inches of rain received in these parts of the country would be sufficient to allow a considerably heavier hookworm infection to develop than actually exists. Drainage, of course, is another factor to be considered in connection with the

hitherto recorded, on the other hand it is possible that the eggs belong to a free-living coprophagous nematode which entered the stool from the soil, and produced numerous eggs in the interval of time before the stool was examined. There are, however, two facts which make the latter hypothesis appear improbable. In the first place, no similar case has been observed in over 7,500 stool samples examined by us in other parts of India, nor has it been recorded by any other observers in India, although tens of thousands of stool samples have been examined by various investigators though not in the particular section of India where four of these five cases were found. All of these stools were deposited on soil which at the time was dust dry, and the samples were collected within two or three hours of the time they were passed. In the second place, if these nematode eggs belonged to a free-living coprophagous species, they should have developed into adult sexual forms in the cultures, and this they entirely failed to do, rhabditiform larvæ, but no other forms, were observed in the cultures for 14 days after they were started. Kobayashi (1920) described cases of *Rhabditis hominis* in school children in Japan, and subsequently Sandground (1925) discovered what appeared to him to be the same organism in nine cases in Southern United States. The offspring of this species, however, like *Strongyloides intestinalis*, appear in the fæces as embryos, not eggs, and furthermore they readily develop into adult sexual forms in all sorts of cultures. Sandground produces good evidence to show that this species is not a human parasite but a coprophagous invader of the stools. Our cases are certainly not *Rhabditis hominis*, since the eggs as passed are for the most part in a very little-developed condition, and besides they do not develop into adults in culture. The eggs certainly resemble those of free-living *Rhabditis*, and it is possible that it is a species of this group which has adapted itself to life in the human intestine. *Heterodera* eggs were found in many places, especially in Bombay.

#### HYMENOLEPIS INFECTIONS

*Hymenolepis nana* appears to be very generally distributed over this entire area, just as it is in north-western India. The local abundance, however, is extremely variable in different villages in all probability due to the habits of the people with respect to the keeping of cooked food overnight in such a way that rats can get access to it. That rats and mice are the source of the infections is very strongly indicated by the distribution in India, as I have pointed out in a separate paper (Chandler, 1927). The highest incidences of infection were found at Ujjain (19 per cent), Bhopal (18 per cent) and Jubbulpore (15 per cent). In one village near Hyderabad 11 per cent were found to harbour *H. nana*, while in another village only 30 miles distant not a single infection was found in 48 examinations. Near Nagpur no infections were found in 96 people examined. The only other places where no infections were found were Bhayndar near Bombay, and Dharwar, significantly enough these were the only places where *Ascaris* infections were prevalent. This bears out our observations in other parts of India that the distribution of *Ascaris* infections and of *H. nana* infections are directly

infections, and 36.1 per cent at Bhayndar, which had 52 per cent of *Ascaris* infections. The situation at Dharwar is extremely unusual, for as a rule the incidence of *Trichuris* infections is more or less in proportion with the *Ascaris* infections. The occurrence of 82 per cent of the latter accompanied by only 1 per cent of *Trichuris* cases is a condition which has been found elsewhere only in neighbouring places in Mysore and clearly demonstrates that in spite of the fact that the two infections are transmitted by food or water contaminated with the eggs of the worms, the conditions favouring one may not be favourable to the other. We have found some evidence for this in other places also, but the difference in incidence of the two infections was nowhere else so pronounced.

Nearly everywhere drinking water is derived from wells, either draw-wells or step-wells, but in either case they are as a rule well-constructed and have raised edges, so that there is little or no possibility of gross pollution. The few *Ascaris* infections found are probably due to the occasional drinking of polluted water from sources other than the wells or to the occasional ingestion of eggs while washing out the mouth with water of tanks, streams, or ponds in the course of bathing. In most places the wells are of sufficient depth to contain water throughout the year, but in some villages the wells dry up in particularly dry years, and then holes are resorted to in the course of streams which are dried up except for occasional low places, sometimes special holes are dug. This, however, does not lead to any great amount of infection, since in the wet season the streams are flowing and gross pollution is carried away, and when resorted to as a source of drinking water there is no rain to wash the faecal material into the holes which are used.

At Bholakpur (Hyderabad) where 22 per cent of *Ascaris* and 3.3 per cent of *Trichuris* infections were found, I was not able to trace the source of the infection. Here water was piped to the village from the city water supply. It is possible that this supply may be polluted, but it seems much more probable that some local source of polluted water is used during the rainy season. The pipe water had been in use for about four years, and it is possible that many of the infections found were acquired previous to its introduction, practically nothing is known of the length of life of *Ascaris* in human beings.

At Bhayndar near Bombay water was obtained from a well adjoining a ground tank with raised earth banks, and the water of the tank and of the well were continuous underneath. There was, however, no evidence of pollution around the banks of the tank and I think it very unlikely that the well was the source of the infections. This well was situated about 600 yards from the village, and it is very probable that when there is abundance of water in the rice fields all around of the village, water is used from sources nearer at hand.

At Dharwar the source of the *Ascaris* infections appeared at first rather mysterious, assuming that it was water-borne, as our observations elsewhere have led us to believe firmly. The majority of the people obtained their water from a large lake-like reservoir which is used as a source of

prime importance in determining the amount of infection in this part of India, since in most places the habits of the people are highly favourable for the propagation of hookworm. The Arabian Sea monsoon current drops most of its rain on the west slopes of the Western Ghats, thus limiting the area of heavy rainfall to a comparatively narrow strip. The Bengal current supplies a moderate and dependable rainfall to the eastern parts of the Central Provinces. In the intervening areas the rainfall is too low and too uncertain to permit an appreciable degree of hookworm infection to develop.

Indiscriminate soil pollution is practically universal. Except in the southern part of the Bombay coast the villages are built compactly and the defæcation areas occur all around their edges. In the southern Bombay coast, however, the houses are scattered so that each household has its own defæcation site. This reduces the total amount of infection in the community and tends to bring about very uneven degrees of infection in different individuals. If the villages in this region were compact like those in other parts of Bombay and Central India, a considerably higher degree of infection than actually exists would be expected.

The nature of the soil exerts a very marked influence on the amount of infection, black cotton soil, which prevails over a large part of the Central India Agency and the western parts of the Central Provinces, is a particularly unfavourable soil for hookworm larvæ, and, at least where the rainfall is light and irregular, hookworm infections will probably never be found to be of any consequence in regions possessing this type of soil.

*Ascaris* and *Trichuris* infections are also negligible except on the southern Bombay coast. Here nearly every household has its own well, poorly constructed without raised edges, so that gross pollution from surface washings results during the heavy rains. The presence of abundant *Ascaris* infection with almost complete absence of *Trichuris* infections in a village near Dharwar is a very unusual and interesting condition. *Hymenolepis nana* infections occur throughout the area, and are locally very common in parts of Central India. No less than seven cases of *Hymenolepis diminuta* infections were discovered in the 1,606 examinations made in this area, four of these were found in 99 examinations at Bhopal, where 18 per cent were infected with *Hymenolepis nana*.

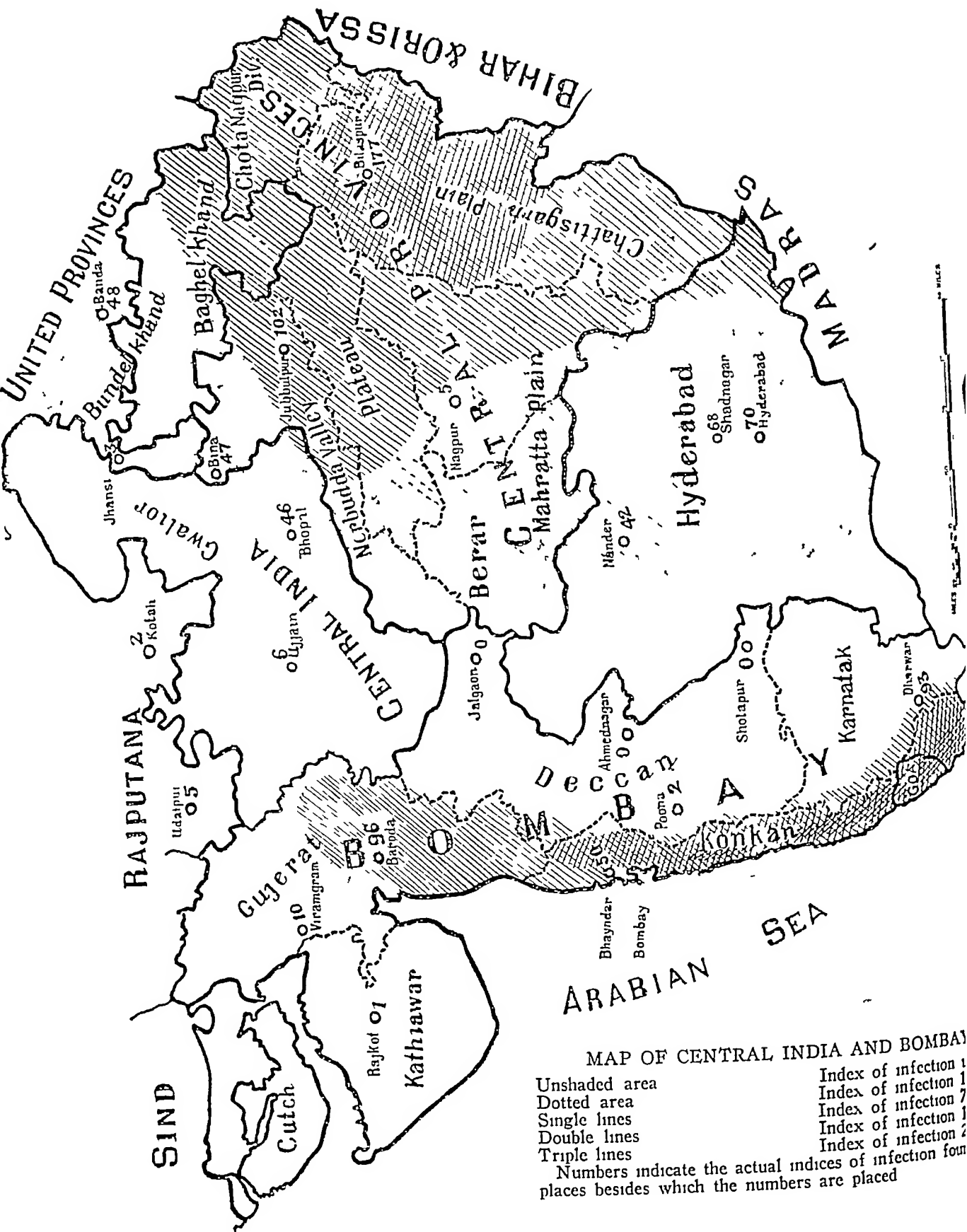
Guinea-worm is locally common where step-wells are used in the western two-thirds of the area here considered. It is especially common in the western states of the Central India Agency, and in the Gujerat and Deccan Divisions of Bombay. *Trichostrongylus* infections occur sporadically everywhere as in other parts of India. One case near Dharwar had numerous rhabditis-like eggs in the stool, which on culturing produced rhabditiform larvæ but no adults, it may or may not represent a true human parasite.

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ches	OCTOBER		NOVEMBER		DECEMBER		ANNUAL	
	Rainy days	Inches	Rainy days	Inches	Rainy days	Inches	Rainy days	Inches
<i>Centr</i>								
Bila 774	24	1.92	07	0.44	05	0.26	63.9	49.63
Jub 790	21	1.68	07	0.46	06	0.26	62.8	55.35
Nag 785	30	2.09	09	0.56	09	0.46	60.0	46.65
Cha 07	26	1.67	09	0.59	07	0.24	60.6	50.22
Ako 570	25	1.85	08	0.45	08	0.50	44.3	30.34
<i>Centr</i>								
Rev 85	22	1.91	06	0.29	08	0.29	56.4	47.91
Bho 18	15	1.02	07	0.47	04	0.30	50.3	43.83
Ujj 55	13	0.77	09	0.64	03	0.07	37.0	27.44
<i>Hyde</i>								
Hy 704	48	3.26	24	1.16	05	0.34	51.7	31.84
Na 77	30	2.22	10	0.58	08	0.48	51.9	37.20
<i>Bomb</i>								
Jalg 561	16	1.11	07	0.53	03	0.12	45.2	29.06
Poo 507	45	4.01	15	1.11	04	0.23	49.4	28.86
Sho 771	41	3.02	15	1.05	06	0.46	40.3	27.55
Dha 41	8.2	5.28	28	1.82	07	0.48	65.8	32.88
<i>Guje</i>								
Bar 543	09	0.91	02	0.09	01	0.04	39.8	34.73
Vir 396	07	0.37	02	0.22	01	0.03	32.5	26.73
Raj 391	11	0.59	03	0.26	02	0.06	30.8	25.84
Bh 177	06	0.54	02	0.08	01	0.04	16.2	13.72
<i>Konk</i>								
Bo 183	27	2.43	05	0.36	01	0.05	75.0	79.38
Rat 165	49	3.52	13	0.92	02	0.08	91.8	96.69
Bha 396	89	7.33	29	2.38	04	0.31	112.2	148.61



or to the tendency of Indians to use water on flooded ground for drinking when an abundance of it is available, or to both, it is difficult to say, but that the heavy rainfall does in some way lead to prevalent *Ascaris* and *Trichuris* infections appears undeniable

#### GUINEA-WORM (*Dracunculus medinensis*)

This worm is common locally throughout nearly all parts of Central India and Bombay, east to about the middle of the Central Provinces. It is said to occur fairly frequently in Damoh District, yet it is said to be absent in Jubbulpore District, even where there are step-wells. It also appears to be absent in the eastern division of the Central India Agency (east of Jhansi District). It occurs in some localities in Wardha and Nagpur Districts and in Berar, but strangely enough is said to be absent in the other western districts of the Central Provinces. It occurs commonly in the western parts of the Central India Agency and in Gujerat, and also throughout Bombay, though it is commonest in the Deccan. Practically the whole of Hyderabad is subject to infection where step-wells are used. Kathiawar has less infection, and Cutch, like Sind, seems to be free. The absence of the infection further east can only be attributed to the non-existence of suitable intermediate hosts, though why the involved species of copepods should be limited to the western half of the peninsula it is difficult to say.

#### OTHER NEMATODE INFECTIONS

*Trichostrongylus* infections were found near Hyderabad (22 per cent), near Rajkot (4 per cent), near Poona (1 per cent), and near Dharwar (9 per cent). Of the 9 cases found in 100 examinations near Dharwar, 8 occurred in Kurbars and Lingayats and one in a Berad, all adults. Only a single *Strongyloides* was encountered, this being at Jubbulpore, but it is probable that it occurs much more frequently than is indicated by this. Of special interest was the occurrence in the stool of a Lingayat female child of 12 years, near Dharwar, of numerous small nematode eggs, 50 to 58  $\mu$  long by 26 to 30  $\mu$  wide, resembling in size and general appearance the eggs of *Rhabditis*. The stool was several days old when examined, and therefore little can be said about the stage of development of these eggs at the time they were passed. Evidently, however, the majority could not have been in a very advanced stage, for most of the eggs seen at examination were in the morula stage, only a few containing developed embryos, which was also true of the hookworm eggs. Portions of the stool were cultured by smearing on the top of a mound of sterile soil in a Petri dish surrounded by water, and also on ordinary nutrient agar. The eggs hatched and the embryos developed into rhabditiform embryos, but never proceeded beyond this stage to produce either filariform embryos or adult sexual forms. The same kind of eggs was found in the stools of three adult females near Anantapur in the Madras Deccan, and in a Christian fisherman in a village near Calicut. I am unable to express an opinion as to the true significance of these eggs in the stools. It is possible that it represents a true human parasite not



80 miles wide in the south, and merging with the Western Ghats in the State of Travancore. The northern two-thirds of the interior, including the whole State of Mysore and the Deccan Division of Madras, as well as the northern parts of Coimbatore and Salem Districts in the south, is an elevated plateau, in most places varying in height from about 1,500 to 2,500 feet, this is a portion of the great Deccan plateau of Central India which also includes a large part of Bombay Presidency and the whole of Hyderabad State. The southern part of the interior is a region of broken groups of hills and intervening valleys and plains.

Three monsoon currents affect this area. The south-west monsoon beginning in May and extending to September affects the west coast and to a less extent the whole southern portion of the peninsula. This south-west or Arabian Sea current sweeps up from the south-west and, striking the Western Ghats, precipitates a very heavy rainfall. After having passed the Ghats very little moisture is left and consequently the centre of the peninsula, right down to the southern tip, receives very little rain from this current, and the northern parts of Mysore and the Deccan Division of Madras still less.

The south-east monsoon, beginning about the middle of June and lasting until early in September, sweeps north-eastward across the Bay of Bengal leaving little or no rain on the Coromandel Coast. From the mouth of the Godavari River to the head of the Bay the amount of rain becomes greater and greater. A certain amount of rain is carried inland through the Agency Division of north-east Madras to the eastern and southern parts of the Central Provinces.

The north-east monsoon becomes active as the other monsoons die, and brings a fairly heavy rainfall to the Coromandel Coast from the middle of Nellore District to the south of Tanjore District. A fair amount is precipitated in a belt 25 to 60 miles wide inside the Eastern Ghats, whereas the centre of the peninsula gets very little. Its precipitation is, however, never as great as that of the south-west monsoon. Although there is usually an interval of about a month between the two monsoons which affect southern India (the south-western and the north-eastern), they come near enough together so that frequently the ground is kept moist in favoured spots for the greater part of the time from May to November. Tinnevely and Ramnad get fair rainfalls only in October and November, from the north-east monsoon, and very little from the south-west earlier in the season. Coimbatore and southern Mysore get a moderate amount from both while the southern part of the west coast gets a large amount from both.

The people of South India are almost entirely Dravidian and nearly 90 per cent are Hindus. Telugus inhabit the north-eastern parts of the Presidency while the majority in the south are Tamils. On the north of the west coast the inhabitants are Kanarese, and further south Malayalam. Primitive hill tribes inhabit some of the mountainous districts of the south-west.

Rice is the most important single crop in the south. Where the rainfall is sufficient, this crop is grown on non-irrigated ground whereas in the drier central parts of the peninsula irrigation is very extensive. In the Deccan tablelands of Mysore and northern Madras there are very extensive areas under wheat, millet, ragi and in some districts cotton. There are extensive forests in the mountains,

opposite, although where small percentages of both occur, they occur together in as many cases as would be theoretically expected. Seven cases of *Hymenolepis diminuta* were found, 1 near Jubbulpore, 4 near Bhopal (both places with very high incidences of *H. nana* infections) 1 near Viramgram, and 1 near Hyderabad.

#### OTHER INFECTIONS

No fluke infections were found anywhere in the area. *Tænia* eggs were encountered in three cases near Jubbulpore, but not elsewhere.

#### SUGGESTION FOR THE REDUCTION OF HELMINTHIC INFECTIONS

The degree of hookworm infection is so light throughout all of this area except the Konkan Division of Bombay that no special measures for its reduction appear to be necessary, the climatic conditions take care of the situation. On the southern Bombay coast education in the method of propagation of hookworm infections and in the injury resulting from them should be undertaken, and an attempt made to get the people to adopt some simple type of latrine, or at least to utilize logs, roots of trees, or other similar places for standing on during defæcation. Along the Malabar Coast latrines are by no means uncommon among the better class of people, and it is quite possible that in time some progress along this line can be made.

The amount of *Ascaris* infection, also, is not sufficient to be worthy of much consideration except on the southern Bombay coast. Here the wells are for the most part not protected by raised edges, and must be grossly contaminated by surface washings during heavy rains. Demonstration of the prevalence of *Ascaris* infections by treatment of a group of school children would in most cases make a sufficient impression so that some effort would be made to protect the drinking water sources from surface washings during the rains. Certainly one of the most important sanitary problems in India is education of the people in the importance of protecting drinking water from pollution. A Brahmin would not accept a cup of boiled water from the hand of an outcast, yet in most cases he would not hesitate to drink water from a tank or well grossly polluted by the fæces not only of himself and his family but also of other members of the community.

*Hymenolepis* infections are sufficiently common throughout Central India to be worthy of consideration with a view to prevention. Suggestions on this point have already been made in the report on North-west India.

#### SUMMARY

Hookworm infection is extremely low throughout the greater part of Central India and Bombay. There is a small area in the eastern part of the Central Provinces which has a moderate degree of infection, while there is every reason to believe that the amount of infection is fairly high in the southern part of the Konkan District of Bombay. Climatic conditions are of

In spite of the extremely high incidence of hookworm infection, 97.1 per cent harboured worms without appreciable symptoms, and even of the coolies emigrating for the second time 94 per cent were in good or fair health. Seventy-eight per cent of returning coolies emigrate back to the Straits Settlements within a year, of these 95 per cent were in good health. In three of four post-mortem examinations made, less than 30 worms were found, the fourth one had 20 necators, but was in good health. The evidence from this, therefore, is that although the incidence of infection is high the degree of infection is low and the effect on health apparently very slight in contrast to the reports from other countries, where heavy and injurious infections are found among this same class of people. The good health of re-emigrating returned coolies indicates that however much they may suffer from increased infection in such countries as Malaya and Ceylon, the effect wears off very quickly after residence in India, this bears out our evidence (Chandler, 1926) that hookworms are lost very quickly when re-infection is checked. This work at Negapatam was the first direct evidence that infection in Indian coolies is less at home than it is after emigration to countries with more favourable conditions for the propagation of hookworm infections. It may be stated here that all subsequent work has borne out this idea so that now it can be accepted as an indisputable fact that the Indian coolies who develop heavy and highly injurious infections abroad are for the most part lightly infected and practically immune from symptoms in their own country.

From 1917 to 1919 the ankylostomiasis inquiry in Madras was transferred to Dindigul. Here an equally high incidence of infection was found. In 12 post-mortem examinations, the average number of hookworms harboured was found to be 11 in the upper and middle classes, 21.2 in police constables and 127 in sweepers. *Ascaris* infections were found in 37.3 per cent and *trichuris* in 11.9 per cent. In villages near Dindigul, also, an incidence of 100 per cent was found, with 20.4 per cent *ascaris* and 7.6 per cent *trichuris*.

Work was carried on in the Trichinopoly Jail from 1917 to 1923. Here a total of 7,930 new arrivals were examined, representing nearly every district in Madras. A total of 97.6 per cent were found infected, the incidence did not fall below 87 per cent in the individuals from any one district. The *ascaris* incidence was 42.7 per cent, *trichuris* 26.2 per cent, and strongyloids 8.2 per cent. Here some illuminating work on the number of worms harboured was done by counting worms after treatment. In Bellary and Anantapur Districts the average worms were only 6.2 and 8.2, respectively, in Tinnevely and Ramnad 21.2 and 24.6, respectively, in Madura, Coimbatore, Salem and Trichinopoly between 28 and 41, in Tanjore 92.5, and in Malabar 102.8. In Coimbatore Jail also high incidences of infection were found.

In 1921 some investigations were made on tea estates in the Annamalai Hills in south-western Coimbatore, where practically 100 per cent infection was found. Worm counts after treatment of 174 individuals showed an average of 92.9 worms, the largest number being found among the low class Panchamas of Coimbatore District. All of this work has been summarized in a single paper by Mhaskar (1924).

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Summary of Examinations made by Dr. Kendrick at Mandapam Camp and in Various Districts in Madras

District	Place examined	Number examined	Per cent hookworm	Average e p g	Ascaris per cent	Trichuris per cent	Tapeworm per cent	Per cent with over 600 e p g
Chingleput	District	651	79	735				
"	Mandapam Camp	366	90.2	584	46	21	0	36
Coimbatore	"	487	80	375	13	4	3	19
Madura	"	1,886	85	522	43	19	0	25
"	District	6,617	62.4	303	14	2	0.6	28
Malabar	Mandapam Camp	48	90	770	54	48	0	52.5
"	District	3,080	81	863	76	58	0	38
North Arcot	Mandapam Camp	497	86.7	425	35	18	1	27
Pudukottah	"	101	74	792	31	18.4	1	
Ramnad	"	1,684	87	446	43	28	1	26
Salem	"	4,374	85.6	382	21	9	1	18
South Arcot	"	1,105	88.7	447	30	14	0.6	27
Tanjore	"	477	88	685	25	13	0	34
"	District	4,520	58	329	6	4	0.3	26
Tinnevely	Mandapam Camp	1,369	83.7	481	56	26	0.3	23
Travancore	"	44	95.5	866	77	41	0	57
Trichinopoly	"	3,188	85.6	486	32	14	0.5	24
South Kanara	District	2,471	69.3	293	91	82	0	15
Chittoor	"	2,124	68.9	483	0.3	0.2	0	29
Cuddapah	"	494	37.9	93	33	15	1	29
Wynaad Estates	Estates	240	87.5	720.0	56	51	0	

# THE PREVALENCE AND EPIDEMIOLOGY OF HOOKWORM AND OTHER HELMINTHIC INFECTIONS IN INDIA

## Part XI.

### MADRAS PRESIDENCY AND SOUTHERN STATES

BY

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THE Madras Presidency with the Native States of Mysore, Sandur, Coorg, Cochin and Travancore covers an area of about 184,000 square miles, a little less than  $2\frac{1}{4}$  times the area of Bengal. It has a population of 53,922,000, which gives an average density of about 293 per square mile. The greatest density is in the districts and states of the west coast where it averages about 475 per square mile, and lowest in the Agency Division (the interior of the northern part of the East Coast) where it averages only 75 per square mile. The East Coast South also has a fairly heavy population of 442 per square mile. The Deccan areas of Mysore and Madras average about 150 per square mile, and Coorg 104.

On the west coast there is a narrow coastal strip 25 to 50 miles wide bounded by the mountain range known as the Western Ghats, rising to an average height of between 3,000 and 4,000 feet, with occasional much higher peaks. Except for a comparatively narrow pass in South India these mountains extend in an unbroken series from the northern part of Bombay Presidency to the southern tip of the peninsula. A broad spur extends eastward to involve Coorg and parts of Western Mysore, another, the Nilgiri Hills, extends north-eastward in the Nilgiri and northern Coimbatore Districts, leaving a high and wet tableland, known as the Wynnaad, between them and the Ghats proper, still another, the Anamalai Hills, extends eastward into the Madura District. In these spurs of hills and high tablelands, particularly in Coorg and Western Mysore, in the Wynnaad, and in Cochin and Travancore, there are extensive plantations of tea, coffee, and other special products. On the eastern side of the peninsula there is a more broken and irregular and less lofty line of hills, the Eastern Ghats, which leaves a coastal strip of irregular width about 30 to 50 miles wide in the north and from 50 to

north-east monsoon in October. The western part of Hassan District gets a much heavier rain than Hassan itself. Bangalore, on the other hand, at an elevation of about 2,900 feet, gets a fair rainfall in August, September and October, but it has a drier atmosphere and lighter and better drained soil, and therefore, although the incidence of infection is high (about 90 per cent), the infections are very light. Coimbatore and Salem lie at lower elevations south of the Mysore plateau. Both are very dry, Coimbatore has a monthly rainfall exceeding 5 inches only in October and Salem between 5 and 7 inches in August, September and October. Salem itself is more favoured by rain, lying as it does near the foot of the Shevaroy Hills, than are other parts of the district except these hills themselves. As at Bangalore, the light well-drained soil and dry atmosphere, together with a distribution of the rainfall over less than a third of the days in the month, prevents the soil from remaining continuously moist even with 7 inches of rain in a month. Sanitary conditions were worse in the Salem village than in any other place which we inspected in South India, or in fact in any part of India, and therefore the dryness of the climate has a highly beneficial effect so far as hookworm infections are concerned. By reference to Dr Kendrick's table it will be seen that coolies from Coimbatore and Salem Districts have lower average eggs per gram and a lower percentage of infections of 600 or more eggs per gram than any others examined at Mandapam.

Near Ootacamund, at an elevation of 7,500 feet in the Nilgiris, we found hookworm practically absent in spite of favourable habits and 8 months of the year with from 5 to 8 inches of rain. The village studied, however, was situated on a hill-side from which the rain would run off very rapidly. Furthermore, the soil was very light, the atmosphere dry, and the temperature sufficiently low seriously to retard larval development, so that a longer period of continuous moistness would be required.

On the west coast we studied villages at Mangalore, Calicut, Ernakulum, Trivandrum, interior Travancore villages, and coolies from several estates in the Wynaad area. The entire west coast, up to a little distance over the crest of the Ghats, is a country of very heavy rainfall, dense forests of cocoanut and other trees, and heavy jungle. In the South Kanara District in the north most of the rain falls in June, July, August and September, but in southern Malabar and Cochin the rains of the south-west monsoon break early in May and continue through October, and in many parts of Travancore they begin in May and extend to near the end of November, with only a partial break in August and September. As one would expect, such a long rainy season, accompanied by favourable soil conditions, high humidity, and abundant shade, leads to comparatively heavy hookworm infections. The latter are, however, somewhat modified by the nature of the villages, as discussed in the following section, and consequently it is only in especially favourable situations for the propagation of the disease, as in especially densely clustered villages or in coolie lines on estates, that the degree of hookworm infection reflects the full benefit of the favourable climatic conditions. The beach villages, consisting mainly of fishermen, have relatively low degrees of infection due to the habit of using the beach for defæcation. At Calicut, for instance, the average eggs per gram among the fishermen on or

and on the west coast there is a very important industry in cocoanuts, in fact the whole west coast where not cleared is densely forested with cocoanut trees

### HUMAN INFECTION

Madras Presidency, unlike any other part of India, has been the scene of very extensive investigation into the prevalence and importance of hookworm infection. Interest in the infection in this part of India arose largely from the fact that a very high proportion of coolie labour exported from India to other parts of the world, particularly Malaya and Ceylon, are Tamils from South India, and after transportation these labourers were found to suffer severely from heavy hookworm infections. It was assumed that the infections were brought from India, and that therefore an investigation into the problem in this country was not only of national but of international interest. The International Health Board of the Rockefeller Foundation became interested, as did the Government of India, and a grant for investigation in Madras Presidency was made by the Indian Research Fund Association in 1916. Prior to that time investigations had been made in a number of jails and hospitals, which showed a relatively high incidence of infection as compared with other parts of India by the simple smear method of examination then in use.

The first investigation under the auspices of the Indian Research Fund Association was made in the emigration depôt at Negapatam where large numbers of coolies from many parts of South India were being exported for labour on estates in foreign countries. In 1916, when the work was started, 41,536 coolies with 2,056 infants passed through the depôt, the average stay being only 48 hours. The stop at Negapatam, therefore, could have practically no effect on the hookworms harboured, and a fair indication of the amount of infection in various districts and states could therefore be obtained from an examination of these coolies. Dr. Mhaskar was placed in charge of the field work.

The results of investigations made at the Negapatam depôt and in other places in Tanjore District were as follows —

	Depôt (7,477 exam)	Negapatam town (1,455 exam)	Village Swamimalai (144 exam)	Village Tiruna Geswaram (95 exam)
Hookworm	98.5 per cent	91.4 per cent	100 per cent	100 per cent
Ascaris	60.4 "	70.4 "	52.8 "	39.9 "
Trichuris	34.4 "	44.8 "	34 "	41.6 "
Strongyloides	7.2 "	8.3 "	0	0
<i>Tænia solium</i>	0.5 "	0.7 "	0	0
<i>Tænia saginata</i>	0.6 "	0.2 "	0	0





In 1925 an extensive investigation of hookworm infection was begun at Mandapam Camp, which is the point of embarkation for coolies going to Ceylon. Dr J F Kendrick, of the International Health Board, who had been active in the earlier work in South India also, took over direct control of both the field and laboratory work which was being conducted jointly by the International Health Board and the Madras Government. The importance of determining the degree as well as the incidence of infection having been demonstrated in the meantime, and a practical and sufficiently reliable method of determining the degree of infection in communities by egg counts having been worked out by Stoll, Dr Kendrick renewed his investigations by the egg count method. From September 1925 up to the end of 1926 over 35,000 egg counts were made. Of these 15,621 were made on coolies arriving at Mandapam Camp from various districts in South India, the rest were made on the general population in some of the districts themselves. With the kind consent of the International Health Board and of the Director of Public Health of Madras, Dr Kendrick has provided me with a complete record of his findings in the Madras Presidency, although they have not yet been published. A summary of the findings is given in Tables I and II. It should be noted that in this table the percentages of infection are low as compared with ours because it represents only those individuals positive by the egg count method, whereas our tables include those negative to egg count but positive to the direct centrifugal flotation method.

It will be seen from a study of the tables summarizing Dr Kendrick's work that the highest average eggs per gram and also the highest percentage of individuals with over 600 eggs per gram are found in Malabar and Travancore on the west coast, next in order come Tanjore, Chingleput and Madura, then North and South Arcot, Ramnad, Tinnevely, Trichinopoly, and Chittoor, then Coimbatore, Salem and South Kanara, and finally Cuddapah. The figures for Pudukottah are not directly comparable with the others since all the individuals examined were in the age groups having the heaviest infections. It will be noted that there is a very marked difference in the Madura figures, and even more in the Tanjore figures, between examinations made at Mandapam Camp and those made in the districts themselves.

This is probably due largely to the fact that the coolies examined at Mandapam are drawn from the very lowest classes, mostly of rural origin, while those examined in the districts are to a large extent better class people, since such people will more readily co-operate in supplying stool samples, and many of them are undoubtedly town residents. Since the coolies remain at Mandapam for an average of only 4 or 5 days, there can be no appreciable change in their hookworms after leaving their homes. There are, however, two other factors which enter into the figures. It is quite possible that not all the coolies who state that they come from a certain district have actually lived in that district during the preceding season. The other factor is the effect of previous residence on Ceylon estates. Between 35 per cent and 40 per cent of the coolies examined have previously been to Ceylon, and Dr Kendrick's analysis of his figures shows that these coolies have egg counts 24 per cent higher than those of coolies who have not previously emigrated. It may be concluded, therefore, that the degrees of

Coast), from the middle of Nellore District to Palk Strait, gets a fair rainfall in October and November and many localities also get falls in August, September and December sufficient to keep the ground moist, the greater part of the time under the favourable conditions of soil and humidity. Our localities near Madras and Nellore fall in this area, and both have relatively high indices of infection. Bezwada is in the narrow area between the part well supplied with rain by the north-east monsoon and the more northern districts which benefit from the south-east monsoon, and consequently has a lower degree of hookworm infection. The south-east monsoon delivers a better supply of rain to the interior parts of Ganjam and Vizagapatam Districts than it does to the coastal areas, due to striking against the hills of the Eastern Ghats. The effect of this is well demonstrated by a comparison of the amount of hookworm at Waltair and at Parlakimedi. The former has an average of 554 eggs per gram and the index of infections is 171, the same as at Bezwada, while the latter has an average of 1,074 eggs per gram and the index of infection is 274. The entire Agency Division of north-east Madras probably has a degree of infection comparable with that of Parlakimedi for, although the population is sparse, the villages are fairly compact and conditions are favourable for hookworm propagation through 5 months in the year.

As stated before, we ourselves made no investigations in the south, central and south-eastern districts of the peninsula, since Dr Kendrick's extensive work among people in and from these parts was sufficient to show how they compared with other districts, and our work overlapped his in other parts sufficiently to show to what extent his results among embarking coolies, and among the general inhabitants of the districts, were comparable with our investigations among the ordinary rural villages. It is evident from Dr Kendrick's figures that Tanjore District has a relatively high degree of infection, and can undoubtedly be compared with Chingleput, whereas Trichinopoly, Madura, Ramnad and Tinnevely occupy an intermediate place between Chingleput and Tanjore on the one hand and Coimbatore and Salem on the other. From a careful study of the climatic and topographical conditions, together with a study of our own and Dr Kendrick's results, we have shown on Map 1 what we believe to be the probable extent of the areas having different indices of infection.

The very large number of examinations made by Dr Kendrick gives very useful information concerning the relative amount of infection of the two sexes in different age groups. Table II is an analysis of Dr Kendrick's result. It will be observed that in both sexes both the incidence and degree of infection steadily increase with age, but less rapidly in females than in males. In the lowest age group the females have more infection than the males, but the males pass the females in the 10 to 19-year group, and continue to become relatively more heavily infected in the higher age groups.

*Necator americanus* is overwhelmingly the predominant species of hookworm affecting man in South India. In 36,685 worms recovered by Mhaskar (1919) from 525 persons treated in the Central Jail at Trichinopoly, 95.3 per cent were necators and 4.7 per cent ankylostomes. The relative percentages found in men from different districts are shown in Table IV. The highest incidences

TABLE II

*Summary of Incidence and Degree of Hookworm Infection in Males and Females of Different Age Groups According to Nearly 30,000 Examinations made by Dr Kendrick in Madras Presidency*

Age	Sex.	Number examined	Per cent hookworm	Average e p g
1—4	male	834	59.1	239
	female	893	61.9	280
5—9	male	2,171	62.9	279
	female	1,752	69.9	307
10—19	male	5,351	73.5	408
	female	2,156	75.7	370
20—50	male	11,526	79.6	508
	female	4,516	83.2	446
Over 50	male	1,290	82	597
	female	468	86	447
TOTAL	male	21,172	75.7	454
	female	9,785	77.4	390

infection found by Dr Kendrick at Mandapam Camp are in general somewhat higher than would be found among the rural people of the districts, whereas his figures for the districts are likely to be a little low for the rural population.

Our own investigations were made at Chitaldroog, Hassan and Bangalore in Mysore State, at Anantapur in the Deccan Division of Madras, at Mangalore, Calicut, Ernakulum and Trivandrum on the west coast, at Ootacamund in the Nilgiris, at Coimbatore and Salem in the centre of the country, and at Madras, Nellore, Bezwada, Waltair and Parlakimedi on the east coast, and in addition we examined specimens sent to us from the Wynaad tea estates by Dr Kendrick's field man. We omitted the south-eastern part of the peninsula since Dr Kendrick's extensive work in this part would have made any work of ours there unnecessary repetition.

Chitaldroog in northern Mysore and Anantapur in Madras are typical of the southern Deccan, where the rainfall is very light and very irregular, and where conditions are very similar to those found in the western part of the Deccan in Bombay. At Anantapur only 4 extremely light infections were found in 100 examinations, all of them under 100 eggs per gram. At Chitaldroog 28 of 75 were found infected, but all but two of these had less than 100 eggs per gram. It is evident, therefore, that in this part of the country hookworm infections are practically absent.

Bangalore, Hassan, Coimbatore and Salem are all situated in the north central portion of South India. Bangalore and Hassan are both on the Mysore tableland, but Hassan is on an elevated spur of the Western Ghats just north of Coorg, at an elevation of about 3,100 feet. It is near enough to the western mountains to get a fair rainfall from the south-west monsoon in July and from the

the male portion of the population follow the time-honoured custom of defæcating on any plot of open ground or in some water course unmindful of surroundings, while women and small children retire to the backyard or some small enclosure specially laid apart, and the process of cleaning is left to the sun, the wind, the rain, the fowls, the pigs, and such a body of sweepers as may happen to be maintained by the Local Board' It must be remarked that under the present efficient organization of the Director of Public Health, with his municipal and district health officers, who for the most part appear to be intelligent and earnest men, conditions in many of the larger towns have very materially improved, and there is hope for more improvement in the future. In the villages, however, the situation is less hopeful. Very earnest efforts are being made to improve rural conditions by propaganda, demonstration and governmental assistance, but the progress is extremely slow.

In nearly all villages in South India the defæcation takes place indiscriminately in any available ground near the houses. The arrangement of the houses in villages everywhere in this part of India, except on the west coast, is ideal for the propagation of hookworm infections. Throughout the greater part of the United Provinces and North-west India the houses are built in such compact groups that no available ground is left for defæcation inside the village walls, nor would such pollution be tolerated except in the case of young children, even if some of the adults were willing to commit a nuisance in such extremely public places. In many parts of Assam and Burma, on the other hand, and on the west coast of South India, the houses are provided with large individual compounds and are separated by fields or patches of jungle so that there is often no common defæcation area, each household has a particular bit of jungle or waste land to which the members go. But throughout the rest of South India the houses are arranged in what might be termed loose groups, not sufficiently compact to prevent pollution inside the village limits, and not sufficiently scattered to prevent a mingling of people from different households in common defæcation areas.

As a rule, especially among the lower castes, the males desire very little privacy. When passing through the country on a railway train early in the morning, men can be seen squatting, frequently only a few yards apart, all around the edges of the villages. They usually go a little farther out than do the females, the nearer places being more or less reserved for the latter. The places particularly favoured are open passageways or small clearings in thickets of prickly pear, the bed of dry streams, the sloping banks of wet streams and ground tanks, and around the edges of cultivated fields. Such places anywhere within 75 to 100 yards from the houses are constantly used, so that the ground in these places must be very heavily infested with larvæ when climatic conditions permit their development. The women and children most commonly defæcate very close to home, more often than not within the village limits, in thickets of prickly pear, within the walls of a fallen house or in passageways between or behind the houses, in fact in any place, however near, where temporary privacy can be obtained. Since most of the men leave the village or congregate in certain parts of it during the later hours of the morning, such temporary privacy is very easy to obtain. In many villages in South India there are special enclosures, provided with rubble,

In the villages studied are situated

Index of Ascaris infection per cent		Trichuris per cent	<i>Hymenolepis nana</i> per cent	Miscellaneous
25	13.3	0	5.3	
2	1	1	3	<i>H. diminuta</i> 1 case, Rhabditis (?) 3 cases
90	44.8	0	3.1	<i>H. diminuta</i> 2 per cent, Fasciolopsis 1 case, Oxyuris 1 case
157	57.3	0	0	Oxyuris 1 case, Heterodera 1 case
97	12	5.3	2.7	Oxyuris 5 cases
126	58	17	14	Tænia 1 case, Trichostrongylus 1 case, Rhabditis (?) 2 cases, Oxyuris 4 per cent, Diphylobothrium 1 case, Heterodera 1 case
11	90.6	37.3	6.7	Trichostrongylus 1 case, Oxyuris 1 case
200	96	84	0	Trichostrongylus 1 case, Oxyuris 5 cases
239	100	98.1	0	
314	80.4	84.8	0	
348	81.4	95.3	0	Trichostrongylus 7 per cent, Fasciolopsis 1 case, Heterodera 1 case
198	100	95	0	Oxyuris 3 cases, Heterodera 1 case, Rhabditis (?) 1 case, Fasciolopsis 1 case
210	91.2	79	0	Oxyuris 1 case, Rhabditis (?) 1 case, Heterodera 1 case
251	73.2	93	0	Heterodera 1 case
264	61.4	57.4	1	Oxyuris 1 case, Heterodera 1 case
284	75	68	1.8	Oxyuris 2 cases
248	78	72	0	Oxyuris 6 per cent
234	48.3	60.7	0	Heterodera 1 case
171	13.3	2.7	0	Oxyuris 5.3 per cent, Heterodera 1 case
171	63	50	3	Trichostrongylus 1 case, Oxyuris 3 per cent
274	56.7	27.8	2.1	Oxyuris 4 cases



near the ocean front were 908 and the index of infection 239, while among the agricultural class living on laterite soil or laterite soil mixed with sand, far enough away from the beach so that the latter would not be used as a defæcation site, the average eggs per gram were 1,810 and the index of infection 314, and this in spite of the fact that the agricultural people live in houses well separated from each other with individual defæcation areas. The village studied at Ernakulum more or less combines the favourable conditions of both the beach and back-country villages in that the defæcation places are for the most part not on an open sandy salt-impregnated beach, and on the other hand the houses are fairly closely situated, allowing a certain amount of intermingling of individuals from different households on common defæcation areas. This Ernakulum village had the highest index of infection, 348, that we found anywhere in South India except in the interior of Travancore. In a beach village near Trivandrum in Travancore, where all the defæcation areas were on pure sand, and where the beach itself was very extensively used, especially by the males, the average eggs per gram were 603 and the index of infection 198. At Mangalore the village studied was not directly on the beach but was on hilly land, with the houses well separated and with large compounds. The shorter rainy season combined with the scattered arrangement of the houses is evidently sufficient to keep the degree of infection well below that of the more southern parts of the west coast. The average eggs per gram here were 700 and the index of infection 200, which is comparable with scattered hill villages with similar climatic conditions in Assam and Burma.

Samples from three tea estates in the Wynaad plateau, together with data concerning the environmental conditions, were kindly collected for us by Dr Kendrick's field men under instructions from Dr Kendrick. The coolies on these estates live in lines similar to those on tea estates in Assam and Bengal, and mingle on common defæcation areas. The average eggs per gram were 890 on the Jessie Estate, 1,371 on the Chundale Estate, and 1,478 on the Arapattu Estate. The amount of infection on these estates is, however, not as different as this might indicate, for the presence of a single infection of 10,000 eggs per gram would add 100 to the average for 100 individuals and 200 to the average for 50. The indices of infection, varying from 251 on the Jessie Estate to 284 on the Arapattu Estate, give a much truer indication of the relative amount of infection on these estates, and also with reference to other places as well.

One feature of the infections on villages in this west coast area is the great variability in intensity of infection, even among neighbours, evidently due to the separate defæcation areas for the individual houses. Among the agricultural people in a village near Calicut, for instance 15 per cent including negatives, had less than 100 eggs per gram, 39 per cent had from 100 to 500, 19.6 per cent had from 600 to 2,000, 13 per cent had from 2,100 to 5,000, and 13 per cent had over 5,000 ranging up to 21,000. On the estates, on the other hand, with common defæcation areas, 65 to 70 per cent fall in the two groups between 100 and 2,000 eggs per gram.

On the east coast investigations were made in villages near Madras, Nellore, Bezwada, Waltair and Parlakimedi. The southern Madras Coast (Coromandel



In the northern parts of South India pigs are seldom found in abundance in the villages, and are frequently absent entirely (e.g., villages studied in Mysore, Anantapur and the north coast areas) whereas in the villages in the more southern areas (e.g., Coimbatore, Salem, Chingleput and the west coast), pigs are usually present, being kept in large numbers in the parts of the villages occupied by the lower classes. From a study of the infection, however, and also from a survey of the epidemiological conditions, it does not appear probable that the devouring of stools by pigs has any appreciable effect on the amount of hookworm infection. The droppings of the pigs containing the undamaged eggs are usually left in the same general localities as the original human stools. The same may be said of dogs. The devouring of stools by cattle and buffaloes may have some beneficial effect, as was pointed out in Part V of this series. The mixing of stools with soil by dung beetles takes place throughout the season when the ground is moist as it does in other parts of India.

Throughout most of South India the soil is favourable for hookworm development, a large part of the country is covered with laterite soil or soil derived from the disintegration of rock, with a considerable admixture of sand in some of the coastal areas. Heavy clay soils and black cotton soil are not common except in parts of the Deccan areas of Mysore and Madras.

The wearing of footgear among the rural people or even in towns is very rare in all parts of South India, and can be neglected as a factor in the epidemiology of hookworm, except among the upper class non-agricultural people of the towns and cities.

#### CLIMATIC CONDITIONS IN RELATION TO HOOKWORM INFECTIONS

As in most other parts of India, the climate, and particularly the amount and distribution of the rainfall, is the dominant factor in determining the amount of hookworm infection in the different parts of South India.

In the general account of the nature of the country in South India a brief description of the three monsoon currents which affect South India was given. It will be clear from this that the amount and distribution of rain in different parts of the country is very unequal.

The north-east coast, from the mouth of the Kistna River northward, gets sufficient rain from the south-east monsoon to keep the ground more or less continuously moist during July, August and early September, and in some parts the latter part of June and the whole month of October also have a sufficient rainfall to keep the ground moist. The southern coast districts get sufficient rain in October, November and December, with September more or less uncertain. In some years sufficient rain is delivered by the south-west monsoon in both August and September to keep the ground almost continuously moist in both these months.

The northern part of the centre of the peninsula (northern Mysore and the Madras Deccan) largely escapes all three monsoons, and therefore has a very uncertain and irregular rainfall, just as is the case in the 'no-man's land' between the south-east and south-west monsoon in some parts of Central India. There is on the average an insufficient rainfall to keep the ground continuously moist in

TABLE IV

*Relative Numbers of Necators and Ankylostomes in Various Districts in Madras According to Results of Treatment in Trichinopoly Jail From Mhaskar*

District.	Number examined	Total worms	Per cent necator	Per cent ankylostoma
Bellary	18	112	96.4	3.6
Anantapur	8	66	97.0	3.0
Salem	10	352	96.9	3.1
Coimbatore	26	902	96.5	3.5
Trichinopoly	116	9,489	97.8	2.2
Tanjore	187	17,278	93.6	6.4
Ramnad	38	935	96.0	4.0
Madura	62	1,780	98.6	1.4
Tinnevely	8	169	95.3	4.7
Malabar	72	7,400	95.1	4.9
TOTAL	545	36,685	95.3	4.7

of ankylostomes were found in people from Malabar and Tanjore, and the lowest in Madura and Trichinopoly. Dr Kendrick informs me that in his extensive experience, also, less than 5 per cent of the total worms are ankylostomes. This is an interesting contrast as compared with North India. In Bengal about 20 per cent of the worms appear to be ankylostomes, and, according to Korke, the latter seem to be actually predominant in northern Bihar. It is quite possible that this will be found to be the case in the United Provinces also.

#### DEFÆCATION HABITS AND OTHER FACTORS INFLUENCING THE DEGREE OF INFECTION

Throughout all of South India the habits of the people are as nearly ideal for the development of heavy hookworm infections as one could find anywhere. Except among the better class people on some parts of the west coast, soil pollution is a universal habit in the villages, and to a very large extent in the towns also. The condition of sanitation in many of the towns of South India has been accurately described by Mhaskar (1924) in the following description of Negapatam: 'Like most of the towns in this part of India, Negapatam is in fact little more than a collection of villages. The state of sanitation is indeed little better than that in ordinary villages where it usually is shockingly bad. Sewage and refuse water is not uncommonly allowed to desiccate in backyards and even in public streets. Privies are found in some of the houses, but seem, oftener than not, to be put to any use except the one they are meant for. The greatest part of

86·7 per cent and the index of infection 97, though probably the extensive irrigation has some effect here

As has been pointed out before, a precarious and irregular rainfall is less favourable for hookworm propagation than a constant one, even if for only a month or two in the year, and this is one reason for the almost complete absence of hookworm in the Deccan. It is also evident, as was shown in a comparison of Dharwar and Sholapur in Bombay, that a light rainfall of 5 to 7 inches is much more likely to allow hookworm propagation if distributed over 10 or more days in a month than if limited to 6 or 8 days, especially if the drainage is good

### OTHER HELMINTHIC INFECTIONS

The coprophagous infections are common in most parts of South India as will be seen by reference to our results shown in Table III and Dr Kendrick's, in Table I. In general these infections are relatively low in the drier parts and relatively high in the wetter parts. Anantapur, for instance, had only 1 per cent each of ascaris and trichuris infection, Chitaldroog 13·3 per cent, and Coimbatore 12 per cent, whereas on the west coast the incidences are from 80 to 100 per cent. Dr Kendrick also found the highest incidences on the west coast.

As in other parts of India, the ascaris infections can be correlated with gross pollution of the drinking water. Wherever ascaris infections were high, the drinking water was found to be obtained from ditches, streams or ground tanks into which considerable quantities of faecal material would be washed during the rains, whereas in localities with a low ascaris incidence the water was obtained from wells with raised sides, which could not be polluted in this way. We believe that the partial correspondence of low ascaris infections with dry areas is due only to the greater frequency of wells as a source of water in these places. At the Anantapur village studied, water was obtained by most of the people from wells which could not be grossly polluted, a few of the lower caste people obtained water from a flowing stream at some distance from the village, but, as has been pointed out in preceding sections, such flowing streams, even when reduced to a series of semi-stagnant pools, cannot be a prolific source of ascaris or trichuris infections. At the Chitaldroog village, also, water was obtained for the most part from wells, though at times some of the people resorted to polluted tanks. At the Coimbatore village, also, nearly all drinking water was derived from wells. At Bezwada, which is the only other village we found with an incidence of less than about 50 per cent ascaris infections, there were a number of well-constructed private wells, but water was also taken by the lower caste people from a canal at one side of the village. During the rainy season the water in this canal has a good flow and when it subsequently becomes reduced to semi-stagnant pools there is little opportunity for faecal material to wash into it. At the Bangalore village which had just under 50 per cent ascaris infection the water used was unfiltered water from a tap connected with the Bangalore City supply carried from a distant reservoir. This, however, had been in use only a short time and a number of wells without raised edges were previously used, in fact were still

Locality	SEPTEMBER		OCTOBER.		NOVEMBER.		DECEMBER		TOTAL	
	my ys	Inches	Rainy days	Inches	Rainy days	Inches	Rainy days	Inches	Rainy days	Inches
<i>Deccan—</i>										
Anantapur	77	5 98	5 8	3 85	27	1 66	0 5	0 17	34 0	21 31
Chitaldroog	73	4 20	7 2	4 61	3 2	2 23	0 7	0 37	47 9	24 70
Cuddapah	86	6 77	6 5	5 36	49	3 44	1 3	0 79	44 4	31 61
<i>North centre—</i>										
Hassan	76	3 70	9 4	6 28	45	2 88	1 0	2 9	66 9	33 74
Bangalore	94	7 35	8 9	6 18	43	2 42	1 1	0 68 0 40	57 7	36 05
Ootacamund	14	5 69	13 8	8 12	85	4 96	3 7	1 77	99 7	52 14
Coimbatore	35	1 51	9 4	6 27	69	3 80	2 5	1 08	43 9	22 08
Salem	88	6 63	9 6	6 87	58	3 47	2 0	0 95	60 6	38 86
<i>South centre—</i>										
Madura	65	4 78	10 7	8 04	74	5 04	3 6	1 98	50 4	34 18
Tinnevely	30	1 85	9 2	7 27	11 2	8 16	6 9	5 07	44 5	30 49
Trichinopoly	64	4 94	9 5	7 09	84	5 64	4 4	2 59	45 8	33 13
<i>West coast—</i>										
Mangalore	60	11 10	10 3	7 76	44	2 27	0 06	0 46	116 9	129 14
Cahcut	21	8 38	11 7	10 31	61	4 90	1 6	1 06	115 0	118 56
Ernakulum	34	9 38	14 9	13 55	77	5 08	2 0	1 53	129 5	111 73
Trivandrum	77	4 14	12 2	10 58	95	6 60	3 6	2 43	94 8	64 40
Kottvam Division of Travancore	39	10 19	15 3	13 89	100	8 11	2 9	2 20	134 6	122 87
<i>East coast—</i>										
Tanjore	71	5 84	8 9	6 20	90	7 40	6 0	4 56	50 1	37 17
Madras	74	4 86	9 9	11 15	109	13 61	5 1	5 35	57 1	49 57
Nellore	60	4 30	8 5	9 85	89	11 78	3 4	3 28	43 9	39 30
Bezawada	95	6 04	6 8	5 20		2 61	0 6	0 35	52 3	35 10
Waltair	87	6 82	7 9	8 91	37	4 02	1 0	1 13	49 6	38 66
Parlakimedi	24	9 61	7 5	6 52	25	1 90	0 8	0 88	68 7	50 03

having ascaris infections, whereas 25 per cent of the coolies from Tanjore District at Mandapam Camp were found infected, and in two Tanjore villages Mhaskar found 40 per cent and 53 per cent infections. This low incidence of ascaris in Dr Kendrick's Tanjore District examinations strengthens our belief that the majority of these were town residents and people of upper classes, which we suggested to account for the relatively low incidence and degree of hookworm infections.

Examination of Table III will show that in the drier central parts of the country (Mysore, Madras, Deccan, and at Ootacamund) the incidence of trichuris infections is relatively low as compared with the ascaris infections. This is especially noteworthy at Bangalore and Hassan where 45 per cent and 57 per cent of ascaris infections were found without a single case of trichuris. A similar condition was found at Dharwar, just north of Mysore. At present we can offer no explanation for this condition, but it seems to indicate clearly some epidemiological difference in the propagation of the two infections.

It will be clear from what has been said that, except so far as the Deccan and west coast areas are concerned, there is no general geographical distribution of ascaris and trichuris infections, in villages where drinking water is derived from wells protected from surface washings these infections will be found to be low, whereas wherever grossly polluted wells and tanks are used they will be found to be very prevalent.

#### OTHER NEMATODE INFECTIONS

Guinea-worm is sporadically distributed in all the drier parts of the central portion of the peninsula, where step-wells are in common use as a source of drinking water, it appears to be especially frequent in the Madras Deccan, Mysore and the Central Districts south of Mysore. It does not occur on the west coast, and so far as I can find is also absent from the east coast districts, even where step-wells or ground tanks are in use. This absence of guinea-worm from the eastern side of India is a very striking phenomenon, undoubtedly correlated with the distribution of suitable intermediate hosts. Filariasis is extremely common on the west coast, but as we have not included it in the scope of our investigations we are not in a position to discuss it.

As elsewhere in India trichostrongylus infections are widely distributed. We found in all 7 cases in 1,567 examinations. This gives an incidence of about 0.5 per cent, as compared with about 1 per cent in the more northern parts of the country.

Of particular interest was the finding in 6 stool samples of Rhabditis-like eggs identical with those found in a single case at Dharwar and discussed in Part X. Three cases were found in 100 examinations at Anantapur, two in 100 cases at Salem, and one among 79 beach fishermen at Trivandrum. We have nothing to add to what we said in Part X concerning the significance of these findings. Occasionally Oxyuris eggs were found, and a number of scattered cases with Heterodera eggs.

mud, or brickwalls about 4 feet in height, which are constructed for the express use of the females for defæcation. These enclosures vary in size from about 30 feet square up to 50 feet square. These latrines, as they are called, have no standing places provided, they are in fact nothing but definitely circumscribed defæcation grounds. No arrangement could possibly be devised which would be more favourable for the development of soil infestation.

It is a very fortunate circumstance that in villages on the west coast, where climatic conditions are more favourable for the propagation of hookworm infections than in any other part of India except Assam and parts of Burma, the villages are very scattered in nature. The houses are built far apart from each other, usually with intervals of 50 yards or more between them, and often several hundreds of yards, the result being that the members of one household seldom mingle on a common defæcation area with members of another household. As already remarked this results in a very uneven distribution of infection, neighbours living under identical environmental conditions may have hookworm infection varying from less than 10 worms up to over 1,000 worms. Such an irregular distribution of worms is not found in villages of the type of those found in other parts of South India or in North-western India. Furthermore, it is very common for the better class people in this region of heavy rainfall to provide themselves with latrines. These are of similar type to those found in parts of the delta region of Burma, they are simple roofed structures built at the edge of a ditch or stream or occasionally over a pit, into which the stools fall. Pits, however, are not common since they fill up with water in the rainy season and overflow. In beach villages, of which we studied two on the west coast, inhabited mainly by fishermen, the open sandy beach is extensively used for defæcation by the men and children and even to some extent by the women. Often the stools are deposited near the water where they are washed away when the tide rises. Even if deposited behind the usual high-tide line, very little larval development can occur. Even in the rainy season the pure sand, exposed to the unobstructed rays of the sun, very quickly becomes dry between showers, migration into the sand when exposed to dryness and the heat of the sun, and migration back to the surface during rains or at night, must quickly use up the reserve vitality of the larvæ, thus shortening their lives. Furthermore, even with a fair amount of development, the acquisition of infection would be very limited. In most places the beach is broad, and the area used very extensive, so that the probability of standing on a previously used spot in which infective larvæ were present must be very small. If the surface dries to a depth of half an inch to an inch, which it does within a few hours after a rain, the larvæ would either retire to the moist layers or be desiccated and then standing on such a spot would be quite harmless. These beach villages are usually built on sandy soil, and often the defæcation sites even on the side of the village away from the beach used mainly by the women, are pure or nearly pure sand, far less favourable for the development of hookworm larvæ than ordinary soil. It is not surprising, therefore, that the amount of infection in these beach villages is markedly lower than in interior villages, even though the houses are much more compactly clustered.

Reduction of ascaris infections can only come by improvement in the water supplies. Where wells exist they can easily be made proof against gross pollution, and where drinking water is obtained from streams or ground tanks, efforts to prevent soil pollution near them, in places whence the eggs will inevitably be washed into them, will eventually bring results. Education with respect to the danger of allowing food to be contaminated by rats is indicated as a prevention of *Hymenolepis nana* infections.

#### SUMMARY AND CONCLUSIONS

Hookworm infection is very prevalent in all parts of South India except in the Deccan Divisions of Madras and Mysore. In the north central part of the lower peninsula (Coimbatore District, the western half of Salem District, and the southern half of Mysore) the infections are extremely light. In the other parts of the centre of the country and along the coastal strip from the middle of Nellore District northward the infections are moderately light. Moderately heavy infections occur along the foot of the Eastern Ghats in Vizagapatam and Ganjam Districts, along the coastal areas from the middle of Nellore District to Palk Strait, and on the crest of the Western Ghats. Heavy infections occur on the west coast, especially from Calicut south, except in beach villages. Considering the number of people living in the moderately or heavily infected areas there is probably more injurious hookworm infection in South India than in all other parts of India combined.

In all parts of South India except some parts of the Deccan soil conditions are favourable for hookworm development. Except on the west coast the nature of the villages and the habits of the people are such as to permit the maximum of hookworm infection which the climatic conditions will allow to develop. On the west coast the scattered nature of the village houses results in separate defæcation areas for each household, this has some protective effect.

The amount and distribution of the rainfall is the dominant factor in determining the degree of hookworm infections. The longer the period when the ground is kept permanently moist the greater the amount of hookworm under any given set of other conditions. The monthly rainfall necessary to bring this about depends on a number of conditions among which are the nature of the soil, the drainage, the temperature, the humidity, and the number of rainy days over which the total monthly rainfall is distributed. From 5 to 7 inches in a month appears to be the usual critical point. If distributed over less than one-third of the days in the month even 6 inches may not be sufficient, unless the soil, drainage and humidity are particularly favourable.

Ascaris infections can definitely be correlated with gross pollution of drinking water. In the Deccan, where good wells are prevalent, these infections are very low, whereas on the west coast, with a very heavy rainfall and little protection of the drinking water from surface washings, ascaris and trichuris infections are very prevalent. In most other parts of South India the incidence of ascaris infection varies from about 40 to 70 per cent. Trichuris infections are about as common as ascaris on the west and east coasts, but less in the central areas, and

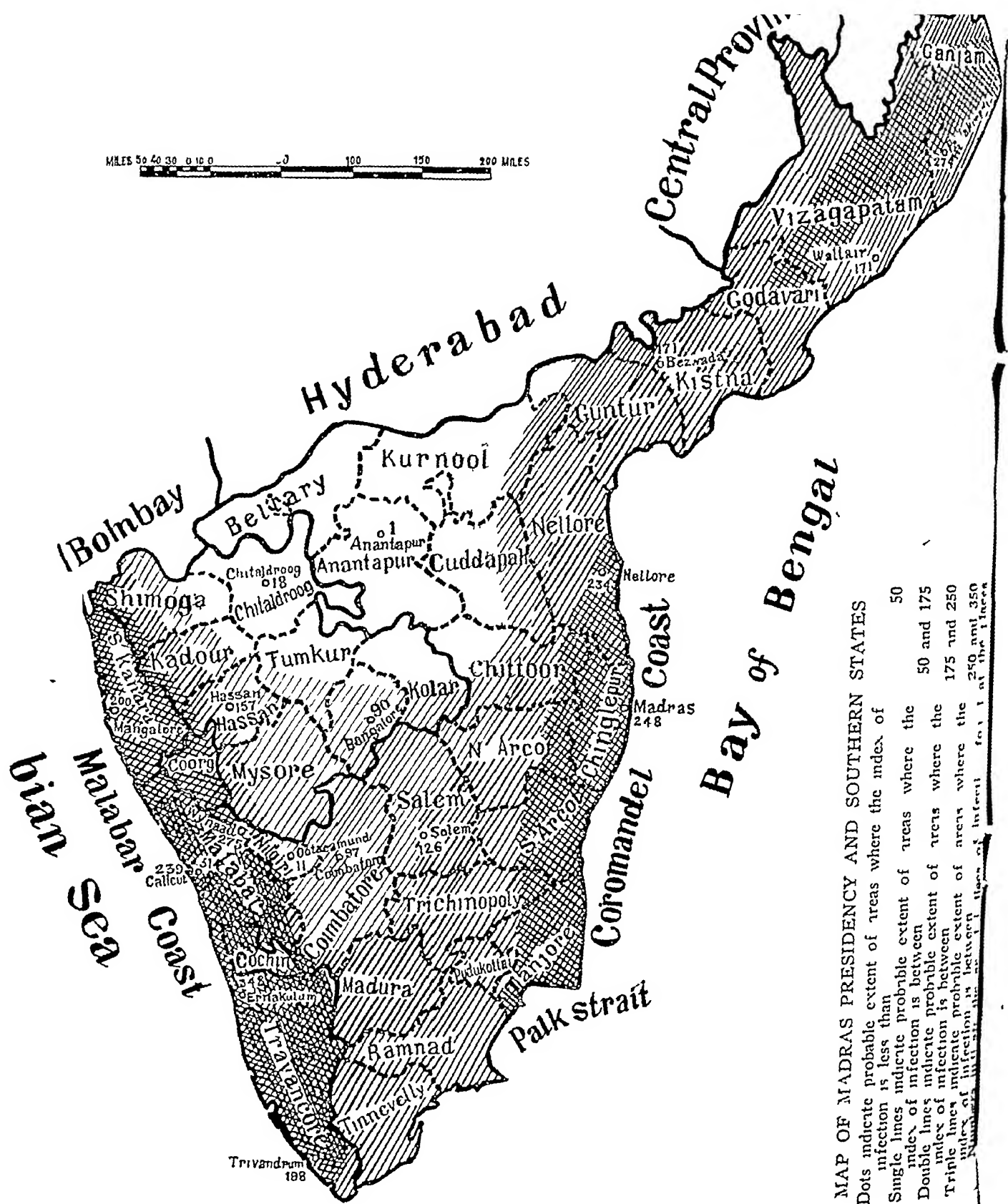
any month of the year, usually September has the largest amount, but even in that month the average fall is only 5 to 6 inches distributed over 7 to 8 rainy days. In the southern part of the interior of the peninsula both the south-west and north-east monsoons deliver moderately light rainfalls so that there are likely to be alternate periods of moisture and dryness of the ground throughout six months of the year.

The northern part of the west coast, South Kanara District, receives a very heavy rainfall from the south-west monsoon during June, July, August and September. Further south (Malabar, Cochin and Travancore) there are heavy falls from this monsoon in May, June and July, and from the north-east monsoon in September, October and November. Although August has little rain, there is sufficient moisture in the ground to keep it from drying between the two monsoons. In consequence the season of moist ground extends from May to January, a longer time than in any other part of India.

The degree of hookworm infection does not correspond so closely to the total annual rainfall as it does to the number of months in the year when the ground remains moist. For instance, Mangalore with about  $5\frac{1}{2}$  favourable months and 129 inches of rain, has notably less hookworm than Calicut or parts of Travancore with 7 to 8 favourable months but a smaller total rainfall. As shown by Chingleput and Parlakimedi even 45 to 50 inches is quite sufficient to allow a heavy hookworm infection to develop so long as the distribution is favourable. When the annual rainfall goes below 40 only moderate degrees of infection occur and below 30 the infection is always very light. When this rain is so distributed that at no time is the soil likely to remain continuously moist for more than a week or two, e.g., at Anantapur, hookworm infections are practically entirely absent, whereas where there are occasional wet periods of two or three weeks several times during the year, a large number of light infections may be present, even though the total average rainfall is less than 25, e.g., at Coimbatore.

The amount of rainfall per month which is necessary to allow hookworm infections to develop differs considerably under different conditions. Where the soil is porous, the atmosphere dry, and the drainage good, e.g., in hilly country, it requires at least 6 or 7 inches of rain in a month. If in addition the elevation is sufficient to make the temperature low enough to retard development it appears to require at least 9 inches. At Ootacamund, for instance, there are 7 months with an average of 5 inches or more and one with over 8 inches, and the total annual fall is 52 inches, yet hookworm is practically absent in spite of very favourable habits for its propagation. Here the temperature is below the optimum, the atmosphere is relatively dry, the soil is porous and the drainage very complete. In contrast Chingleput has an annual rainfall of  $46\frac{1}{2}$  inches with 10 and  $12\frac{1}{2}$  inches in October and November, respectively, and about 5 inches in August, September and December, the temperature is near the optimum, the humidity is high, and the soil is less porous and not well drained. The incidence of infection here was 96 per cent and the index of infection 248. Hassan in Mysore has only  $33\frac{1}{2}$  inches a year, it has less than 7 in every month of the year. Yet here we found 100 per cent infection and the index of infection 157. At Coimbatore, with only a single month with over 5 inches ( $6\frac{1}{4}$  in October) the incidence was





MAP OF MADRAS PRESIDENCY AND SOUTHERN STATES

Dots indicate probable extent of areas where the index of infection is less than 50

Single lines indicate probable extent of areas where the index of infection is between 50 and 175

Double lines indicate probable extent of areas where the index of infection is between 175 and 250

Triple lines indicate probable extent of areas where the index of infection is between 250 and 350

Number of infected persons at the time of the outbreak

being used to some extent. It is also very likely that water was taken by the lower class people from a polluted ground tank. At the Salem village, with 58 per cent ascaris infections, there was a recently discarded well which must have been a prolific source of ascaris infections among the low class people who used it. The substitution of a new well so situated as to be practically free from gross pollution will probably lead to a marked reduction in infection among these people. Polluted tank water was also used to some extent by the people of this village. At Hassan, with 57 per cent ascaris infections (but no trichuris), a large ground tank and also a well subject to pollution were used, at Ootacamund with 90.6 per cent ascaris a step-well subject to very gross pollution was used, at the village near Madras, with 78 per cent ascaris infection, both wells and ground tanks were used, and both were subject to a certain amount of pollution, especially the tank, at villages near Nellore, Waltair and Parlakimedi, with 48 to 57 per cent ascaris infections, polluted step-wells or ground tanks were used, everywhere on the west coast water is commonly derived either from private wells or tanks, or merely from flooded fields, streams or ditches. Most of the houses are provided with their own wells inside the compound, but these wells for the most part are mere holes in the ground lined with brick or stone, and subject to gross pollution from surface washings during the torrential rains, comparatively few of them have raised edges. This is very much the same condition as exists in many parts of lower Burma, where there are likewise very prevalent and heavy ascaris infections. On the Wynaad tea estates the coolies are heavily infected with ascaris and trichuris, just as is the case on every other tea estate which we have examined in India, regardless of the source, or supposed source, of the drinking water. On the Jessie estate there is a well with parapet walls, and no tanks or ditches, on the Arapattu estate pipe water is supplied from a distant reservoir, and on the Chundale estate there is a well which is not provided with a parapet wall. Strangely enough the Chundale estate has the lowest incidence of both ascaris and trichuris infections (61.4 and 57.4, respectively), the Jessie estate has 73.2 per cent of ascaris and 93 per cent of trichuris, and the Arapattu estate with its piped water has 75 per cent of ascaris and 68 per cent of trichuris. We found similar conditions in Darjeeling, Dooars and Assam estates where the water seemed to be beyond suspicion of gross pollution. Either the coolies on tea estates habitually use water from some other polluted source, or else there is some special condition on tea gardens which leads to infection with these parasites. It is a question which seems worthy of careful investigation.

It will be observed that Dr. Kendrick records considerably lower incidences of ascaris infections for corresponding localities than we do. It is probable that this is due in part to the fact that most of the stools were examined only by egg count and not by direct centrifugal flotation. We often find ascaris eggs by the latter method when we miss them by the former. Furthermore, since the main interest in Dr. Kendrick's work centered in hookworm infections it is possible that some of his microscopists may have failed to record all the eggs of other worms which were seen. It is especially noteworthy that of the 4,520 examinations made in Tanjore District, only 6 per cent are recorded as

The patients were not in any way selected but as each sample of the preparation was received, a definite number of consecutively admitted patients were placed under treatment. The first few patients were treated in the early part of 1924, another batch in 1925 and the remainder in the first half of last year, 1926. Many of the patients appeared to be extremely ill but none of them were actually moribund when the first injections were given. The patients were either previously untreated or had received a definite course of treatment and had subsequently relapsed, the latter class are referred to as 'resistant cases'.

*The Diagnosis*—In every case the diagnosis was made by the demonstration of the presence of the parasite by examination of the peripheral blood, by spleen or liver puncture, or by cultural methods.

*Proof of Cure*—As the writer has pointed out before there is no absolute proof of cure in this disease. A clear history that the patient has remained in perfect health for six months after discharge is in the opinion of the writer the only reliable proof of cure and this has been accepted as the final criterion in this series\*. In every instance, however, a spleen or liver puncture with culture was done before the patient was discharged from the hospital and, as the time factor appears to be an important one, an interval of at least 10 days was allowed to elapse after the last injection before the puncture was carried out in all but the first few cases of this series.

In one case the culture showed the presence of leishmania at the time of discharge. This patient has been seen recently, he has shown no further symptoms and is apparently in perfect health.

In a few instances the culture became contaminated.

*Dosage*—A comparatively uniform system of dosage was adopted throughout this series. In the case of adults an initial dose of 0.1 gramme was followed by a second dose of 0.2 gramme and subsequent doses of 0.3 gramme. Children were given proportionately smaller doses but in no instance was less than 0.05 gramme given as an initial or 0.1 gramme as a maximum dose. In a few instances the dosage had to be modified on account of vomiting.

The majority of the patients received a course of 10 or 11 injections but resistant patients and those who could not tolerate the full dose were given a few more injections. As no relapses were occurring amongst the patients who had been given 10 injections, towards the end of the series 8 injections were adopted as the routine treatment for all previously untreated patients who could tolerate the full individual dose.

\* *Note*—A solicited criticism of the writer's paper on Stibosan (No. I of this series) was that 6 months was insufficient time to allow before concluding that the patient was completely cured. This is a point worth accurate investigation, but the writer cannot remember a case of relapse in which the patient did not show very definite signs of relapse within three months. Instances have been observed in which the patient carried on for periods up to a year without returning for treatment, but in these instances the patients always give a history, or showed signs, of having been definitely ill for some considerable time.

## TAPEWORMS AND FLUKES

*Hymenolepis nana* appears to have a rather irregular distribution, it is commonest in the dry Deccan and north central parts of South India, irregularly present in a small percentage of individuals on the east coast, and very rare on the west coast. In the entire 1 567 examinations, 39 cases were found, but 31 of these occurred in 595 examinations in the Deccan and north central areas. The highest incidence was at Salem, where 14 per cent of 100 people examined harboured this parasite, this village, as we have already remarked, was the filthiest of any in which we worked, and was certainly an excellent place for rats to abound. The dirtiness of the habits in other ways would suggest the probability of carelessness in habits with respect to keeping food uncontaminated by rats. The next highest incidence (6·7 per cent) was at Ootacamund which also was a particularly filthy village, affording every opportunity for rats to contaminate food in the squalid and unlighted den-like houses. In the scattered houses of the west coast much less opportunity for harbouring rats is given, and the saturation of the ground outside the houses during the long season of heavy rainfall is also unfavourable for these rodents.

*Hymenolepis diminuta* infections were found in 3 cases, two at Anantapur and one at Bangalore. Only a single tænia infection was encountered, this was at Salem. It appears probable from this that most of the tapeworm cases recorded by Dr Kendrick are *Hymenolepis nana*. One infection with *diphyllobothrium* was found, this also was at Salem.

Three scattered cases of fasciolopsis infection were found, one at Bangalore, one at Ernakulum and one at Trivandrum. No other fluke infections were observed.

## SUGGESTION FOR REDUCING INFECTIONS

There is little to say under this heading which has not already been said in the sections on other parts of India. In the central parts of the country and in the Deccan Division the infection is light enough, so that no special measures are warranted. On the west and east coast, however, there is no question but that hookworm is distinctly injurious. On the west coast some protection is obtained by the fact that the village houses are scattered and have separate defæcation areas, no further reduction seems possible without the development of the habit of using latrines or at least standing places so that the feet will not come in contact with infested soil, and this, unfortunately, will probably come very slowly. On the east coast the conditions and habits are more favourable for hookworm propagation but the climate less so. Here also the development of the use of latrines and standing places seems to be the only possible solution, and the greater compactness of the villages makes this even more difficult to accomplish than under the conditions existing on the west coast. The only hope is a continuous hammering away at the problem, conditions have already been greatly improved in the cities and towns, and eventually this improvement will spread to the villages if efforts to accomplish it are not relaxed on account of temporary failure.

Of the 10 resistant cases in one instance the temperature remained above normal and in two others there was no fever throughout the course of treatment of the remaining 7 the mean number of injections prior to the cessation of fever was 5.7

*Splenic Enlargement*—On admission the average measurement of the spleen of the 58 patients who were eventually discharged as cured was 4 inches below the costal margin, at the time of discharge the measurements were as follows—

Not palpable	21
Just palpable	28
Palpable 2" below the costal margin	3
" 2½" " " "	1
" 3" " " "	1
" 3½" " " "	1
" 4" " " "	1
Size not noted	2
	—
	58
	—

*Weight*—In one case the weight on discharge was not noted, of the other 57, five lost weight and 52 gained weight or were the same weight at the time of discharge as they were before treatment commenced. Of the former group the average loss was 4½ lbs—one patient losing 10 lbs—and of the latter the average gain was 7.79 lbs, the mean of the whole series was a gain of 6.72 lbs.

*The Leucocyte Count on Discharge*—In five instances the count was not recorded, in the remaining 53 it was in every instance over 6,000 per c mm and the mean of the series was 7,545.

*Dosage in Cases in which a Complete Cure was Effected*—Of the 50 patients who were completely cured 9 had previously received treatment and had relapsed, that is to say were resistant cases, and 41 were being treated for the first time.

*Number of Injections*—The details of the numbers of injections given in the individual cases are shown below—

Number of injections	Number of ordinary cases in which given	Number of resistant cases in which given
8	7	
10	19	3
11	7	
12	4	
13	3	3
14	1	
15		2
20		1
	—	—
	41	9

The mean of the whole series is 10.85. The mean number of injections given in the 41 ordinary cases was 10.34 and in the resistant cases 13.22.

practically absent in some areas (e g , Bangalore and Hassan) even though there are numerous ascaris infections

*Hymenolepis nana* occurred to the extent of 25 per cent of all the people examined They were especially prevalent in the drier areas (Deccan and north centre) and practically absent on the west coast

Of other infections 6 cases with Rhabditis-like eggs in the stools were found, trichostrongylus occurred in 05 per cent, *H diminuta* in 2 cases, taenia in 1 case, diphylobothrium in 1 case, and fasciolopsis in 3 cases

Only unrelaxed efforts to develop the use of latrines or standing places in villages can be suggested as a means towards reducing hookworm infections This will probably be more easily accomplished in the scattered villages of the west coast than in the more compact ones in other parts of the country Efforts to prevent gross pollution of drinking water are necessary to control ascaris infections Education with respect to the dangers of leaving food exposed to rats is indicated as a prevention of hymenolepis infections

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1 in 8 patients would relapse after this course of treatment, that is to say, the cure rate for a course of 8 injections is probably not less than 87·5 per cent. Or, to carry it further, one might say that as 26 patients received 10 injections or less the relapse rate with this course is unlikely to be more than 1 in 27 patients, or that the cure rate is probably not less than 96·3 per cent.

On the same principle we might say that in a mixed population of previously untreated patients total doses of 2 grammes and 3 grammes would produce cure rates of not less than 93·75 and 97·37 per cent, respectively, and that total doses of 3 grammes and 4 grammes per 100 lbs body weight of patient would produce cure rates of not less than 93·33 and 97·22 per cent, respectively.

*Complications and Sequelæ*—The only complications that has been noted in this series is vomiting. The frequency of the occurrence of this symptom seems to vary somewhat in the different batches of the preparation that have been used. In a few patients vomiting occurs after the initial dose but in most instances it does not occur until the bigger individual doses are given. It is an indication to increase the size of the dose cautiously, if this is done tolerance can often be established. In only very few instances did this complication prevent the maximum adult dose of 0·3 gramme being administered.

Post-treatment jaundice, such a common sequel to treatment by many of the other pentavalent compounds of antimony, has not occurred as far as it is possible to ascertain in any case in this series.

### *Conclusions*

No. 693 (Von Heyden) is a compound of very considerable value in the treatment of kala-azar. It is a compound of low relative toxicity in mice and it is well tolerated by man in comparatively large doses.

In the first 61 unselected cases in which this compound has been used there have been no deaths and, excluding the two totally resistant cases, no relapse.

With the exception of vomiting there are no disagreeable symptoms associated with the administration of this compound and no sequelæ.

A course of 10 injections, totalling 2·7 grammes of the compound, should produce a cure rate of at least 93·33 per cent amongst average adult Indian patients and there is every indication that a course of 8 injections will produce a very high cure rate.

In a mixed population an expenditure of 2 grammes per patient should produce at least a 93·75 per cent cure rate amongst the patients.

My thanks are due to Prof. Hans Schmidt of Dresden, to Messrs. von Heyden, the manufacturers, and to the Haverro Trading Co., 15, Clive Street, Calcutta, the agents for India, for a generous supply of this compound.

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# THE PENTAVALENT COMPOUNDS OF ANTIMONY IN THE TREATMENT OF KALA-AZAR II NO 693 (VON HEYDEN), AN ANALYSIS OF THE RESULTS OF THE TREATMENT IN THE FIRST 61 CASES

BY

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*The Preparation*—This compound is an amine salt of para-amino-phenyl stibinic acid. It is in the form of a light brown powder and contains about 40 per cent metallic antimony (Uhlenhuth, Kuhn and Schmidt, 1925). It is supplied by the manufacturers in sealed ampoules and in this condition it undergoes no change when kept at room temperature in Calcutta throughout the year. If the ampoule is opened the preparation becomes insoluble within a day or two. It is easily soluble in distilled water and in sterile distilled water it makes a sterile solution. No change appears to occur in the solution if it is kept for an hour or so before administration, but in about 48 hours a white precipitate will have separated.

*Relative Toxicity*—The toxicity of this compound is very low, the minimum lethal dose is more than ten times as great as that of sodium antimony tartrate. The toxicity was tested on white mice, English variety, of an average weight of about 15 grammes. The injections were given intravenously into the tail vein. The results of the experiments were as follows—

Dose in grammes per kilogramme body weight of mouse	Number of mice	Number surviving
150	10	10
200	10	9
250	10	9
300	10	7

*The Patients*—The patients were all 'in-patients' in the Carmichael Hospital for Tropical Diseases; two were Europeans, 7 Anglo-Indians and 52 Indians.



same salt was used. And the senior writer (Napier, 1924) reported 20 deaths amongst 139 cases in which either sodium or potassium antimony tartrate was used. Thus the death rates alone in these three series were 26 per cent, 12 per cent and 14.4 per cent, respectively. The fact that all these patients did not remain to complete their treatment and that certain of them relapsed and eventually died has also to be taken into consideration. All these were hospital patients and were therefore being treated under much better conditions than the majority of the patients who undergo treatment, but on the other hand they were not a fair sample of the infected population as debilitated patients are more likely to seek admission to hospital. Taking all these facts into consideration it seems safe to assume that the eventual recovery rate in cases in which the antimony tartrates are used is not more than 85 per cent and may be as low as 70 per cent.

In Calcutta we find that the hospital patient suffering from kala-azar usually remains in hospital until he is discharged as cured. The dispensary patient, or out-patient, seldom completes his full course of treatment. Their reasons for discontinuing treatment are numerous, some patients are so ill that after having dragged themselves to the dispensary three or four times they give up the struggle, some attend 6 or 7 times without much benefit, become discouraged and do not attend any more, some attend 12 to 15 times, feeling so much better they decide that they are cured and do not attend any more, and others though willing to attend cannot afford the fare to Calcutta. This habit of discontinuing treatment before the course has been completed is a very common practice even amongst patients undergoing treatment at local dispensaries but is much more so in the Calcutta dispensaries where the majority of the patients come from long distances.

As the majority of the quarter of a million odd persons treated for kala-azar in Bengal and Assam during the year 1925 were dispensary patients it seems important that we should have some indication, firstly, what the general recovery rate is amongst all such patients, secondly, what the recovery rate is in 'stopped treatment' cases, i.e., of patients who receive what we consider a totally inadequate course of treatment, and thirdly, as there is no indication at any time during the course of treatment as to whether a patient is cured, or not, and as there must be some limit to the amount of antimony any one patient is given, what percentage cure rate may be expected after various definite courses of treatment, so that administrative officers may advise those working under them as to the course of treatment that should be administered in kala-azar dispensaries.

Investigations on these points might be carried out on a large scale amongst patients who attend the many kala-azar dispensaries in Assam and Bengal, but to do this special records would have to be kept and special enquiries made. The eventual fate of the 'stopped treatment' patient is not usually investigated. The officer who is zealous for the success of his dispensary is liable to pin too much faith in the saying, 'no news is good news,' and to assume that because he has not heard of the death of his patients they have not died, he is also liable to assume too readily that death was due to 'other causes'.

*The Results of Treatment*—The immediate results of treatment in the 61 cases were as follows —

Discharged cured	58
Failed to respond to treatment	2
Failed to complete course	1
	—
	61
	—

*The Failures*—The first was a European male aged 24 years, he had had symptoms of kala-azar for about 15 months but as he had not been in an endemic area for 2 years prior to this date it seems probable that he had been infected for about 3½ years. He was given a course of potassium antimony tartrate injections when the diagnosis was first made, he then received 39 urea-stibamine injections but his condition did not improve. When he was admitted to our hospital his general condition was fair but he was not fit to carry on his work, he had a huge spleen reaching 3 inches below the umbilicus and he was getting a daily rise of temperatures to a little over 100°F. He was given 23 injections of No 693 during a period of 55 days, the total amount of the compound given was 5.1 grammes which corresponded to a dose of 3.6 grammes per 100 lbs of body weight. He did not appear to improve at all except that the temperature did not rise so high and his spleen decreased by about 1 inch. Parasites were still present in large numbers in his spleen at the time he was allowed to leave the hospital. He then went to a hill station and received further antimony treatment, he eventually died of pneumonia about 18 months later.

The other patient was an Indian boy aged about 12, he had previously received 60 injections of sodium antimony tartrate and several of urea stibamine. He was given 15 injections of No 693 over a period of 32 days, this amounted to a total dose of 2.75 grammes, or 4.6 grammes per 100 lbs body weight. He was removed from hospital still suffering from fever and with parasites still present in his spleen. He received further treatment outside, was eventually re-admitted and given another course of antimony treatment without showing any improvement, was again allowed to go out of hospital and has subsequently died.

*Patients Discharged as Cured*—Of the 58 patients discharged as cured I have been able to get into touch with 50, all of these have remained in perfect health for 6 months and have received no further treatment. About the other 8 patients no information is available, in no case is a relapse known to have occurred.

### *The Progress during Treatment and the Clinical Indications of Cure*

*Number of Days under Treatment*—The mean of the number of days under treatment of the 58 patients discharged as cured was 24, of the 48 previously untreated cases the mean was 23 and of the 10 resistant cases it was 29.

*Cessation of Fever*—Of the 58 patients discharged as cured 3 were afebrile and one febrile throughout the course of treatment, of the remaining 54 the mean number of injections prior to the fall of temperature to normal was 4.57.

Of the 48 previously untreated patients one was afebrile throughout and of the rest the mean number of injections prior to the fall of temperature was 4.40.

kala-azar When an ambiguous reply was received a second postcard was sent but otherwise only one postcard was sent to each patient

### ANALYSIS OF RESULTS

The information which we obtained was as follows —

Patients in a good state of health having received no further injections for kala-azar	101 or 58	per cent
Patients who had had fever and subsequently received a further course of injections	36 or 20	7 „
Patients who had died	37 or 21	3 „
	174	

The result of the analysis of these according to the number of injections that they had received is given below (Table I)

TABLE I  
*Number of Injections*

Number of injections received.	Number of patients	Number cured	Number died	Number relapsed	Percentage cure rate
3—5	23	4	10	9	17.4
6—10	26	8	9	9	30.8
11—15	19	11	3	5	57.9
16—20	15	11	3	1	73.3
21—25	19	14	3	2	73.7
26—30	22	14	3	5	63.6
31—35	17	13	3	1	76.5
36—40	18	13	1	4	72.2
41 or more	15	13	2	0	86.7
TOTALS	174	101	37	36	

The 15 cases of the last group should have been spread over three groups but this would have necessitated basing percentages on absurdly small figures,

*The Actual Total Dose*—The patients can be divided into groups according to the actual total dose received, as follows —

	Ordinary cases	Resistant cases
Less than 1 gramme	3	
1 to 149 gramme	8	1
15 to 199 gramme	4	
2 to 249 grammes	13	2
25 to 299 grammes	9	5
3 grammes or over	4	1
	—	—
	41	9

The mean of the total dose given to the 50 patients is 2 19 grammes, to the 39 previously untreated patients 2 06 grammes and to the 9 resistant patients 2 75 grammes

*The Relative Dose*—As in the previous series the doses have been calculated according to the weight of the patient, the 'relative' dose in this series is the dose per 100 lbs body weight of patient. The patients can be grouped according to the relative total dose administered, as follows —

Group	Ordinary cases	Resistant cases
2 to 249 grammes	4	2
25 to 299 grammes	10	1
3 to 349 grammes	15	1
35 to 399 grammes	6	1
4 grammes or over	6	4
	—	—
TOTAL	41	9

The mean of the relative total dose given to the 50 patients is 3 35, to the previously untreated patients 3 31 grammes and to the resistant patients 3 50 grammes

*Dosage in Relation to Age*—The means of the actual and relative total doses in relation to the age of the patient are shown below —

Age group	Number of patients	Mean actual dose grammes	Mean relative dose grammes
Under 10 years	13	1 24	3 81
10 but under 20	15	2 22	3 64
20 or over	22	2 75	2 96

The younger patients were naturally given a smaller actual total dose but there was a tendency to give them a larger relative total dose than was given to the adults

*The Cure Rate*—As all the previously untreated patients in this series were cured it is not possible to prepare a cure rate curve. From a practical point of view one of the most important points to be considered is the number of injections necessary to produce a cure. Seven of the previously untreated patients were given 8 injections only. It would not be safe to assume that this number of injections would cure every patient, as the next patient given 8 injections might relapse. But it would be fairly safe to say that probably not more than

Table III gives the percentage cure rates when the cases are grouped according to the relative total dose

TABLE III  
*Relative Total Dose*

Relative total dose	Number of patients	Number cured	Number died	Number relapsed	Percentage cure rate
0.1—0.5	39	8	16	15	20.5
0.51—1.0	26	12	7	7	46.2
1.01—1.5	15	10	2	3	66.7
1.51—2.0	20	15	1	4	75.0
2.01—2.5	22	14	5	3	63.6
2.51—3.0	15	10	3	2	66.7
3.01—3.5	20	17	1	2	85.0
3.51 or more	17	15	2		88.2
TOTALS	174	101	37	36	

*The recovery rate and dosage during different age periods*—The cases have been divided into three age groups and the cure rates calculated for each. These are given below (Table IV)

TABLE IV

Age period	Number of patients	CURED		DIED		RELAPSED	
		Number	Per cent	Number	Per cent	Number	Per cent.
10 years and under	51	30	58.8	11	21.5	10	19.7
Over 10 but not over 20	61	39	63.9	7	11.5	15	24.6
Over 20 years	62	32	51.6	19	30.7	11	17.7
TOTALS	174	101		37		36	

# THE CURE RATE IN KALA-AZAR

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THE statement is repeatedly made that the introduction of antimony treatment in kala-azar has turned a 95 per cent mortality into a 95 per cent cure rate. This figure is too high in each application. We cannot estimate the spontaneous recovery rate in kala-azar as we have no idea what percentage of the population suffer from a mild clinically unrecognisable form of the disease. The senior writer has had personal experience of 3 patients who had a transitory infection, demonstrated by blood culture, none of these patients had any form of specific treatment, they have been under direct observation from one to four years, they have shown no further symptoms of the disease and subsequent blood cultures have been sterile. If larger numbers of the cases of fever of short duration were investigated in this way it seems very probable that a large number of cases of transient leishmaniasis in man would be demonstrated. Even amongst the cases in which the disease is well-established the natural cure rate is probably not less than 10 per cent.

Again, we have little accurate information about the recovery rate amongst treated cases. Most writers have an unfortunate way of neglecting to report deaths and failures. Even the writers of public health reports are somewhat vague about the deaths that occur during treatment. Certain figures, however, are available. Knowles (1920) reports 22 deaths amongst 86 cases in which potassium antimony tartrate was used, this figure included 6 deaths from influenza. Muir (1919) reported 18 deaths amongst 150 cases in which the

those referring to the higher doses—were based on too small a number of observations

As we explained above, the tendency is for the out-patient to discontinue treatment early whereas the hospital patient will usually remain until discharged. We therefore, turned to the hospital records to supplement our figures for patients receiving longer courses of treatment. The senior writer had already published an analysis of results of treatment of all patients discharged from the Carmichael Hospital during 1922, in order to prepare curves we took the figures

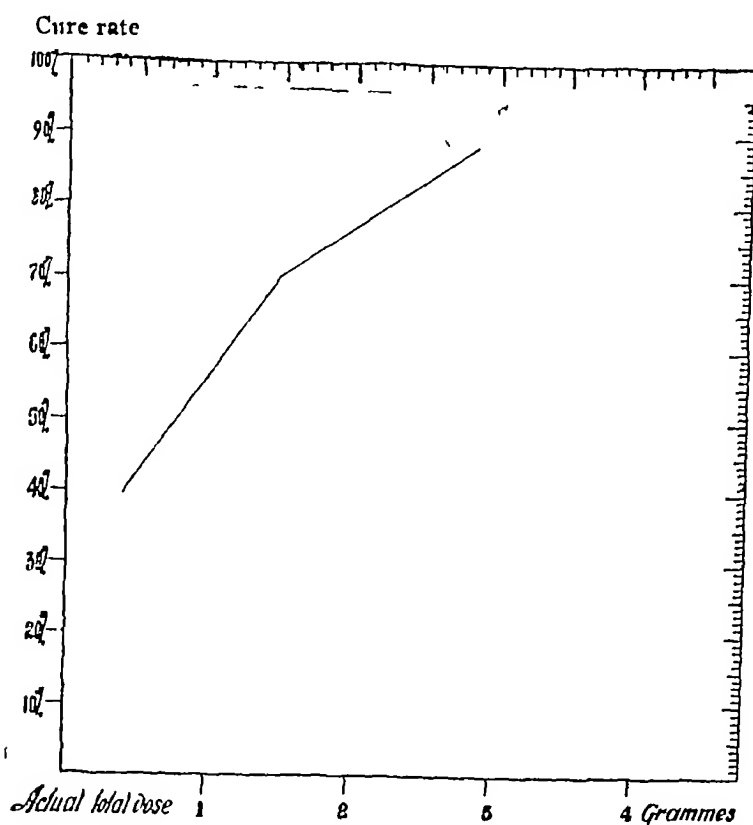


Fig 1—Actual total dose

from this series and after excluding all 'resistant' cases, i.e., of patients who had previously received a full course of treatment before coming to hospital and had relapsed, and cases in which the patient had been admitted *in extremis*, as these would not normally be treated as out-patients, we added the remainder to the out-patient figures already tabulated above. The rule in hospital had been that every patient received 30 injections first of all, after which each case was considered on its merits, if the patient had shown signs of being 'resistant' by the temperature remaining high for a longer time than usual further treatment was given. Thus the 'resistant' cases received more treatment than the normal case and consequently the relapsing cases were more likely to be found amongst those receiving the largest doses. This would cause a misleading fall in the cure rate curve. In order to avoid this effect and in order that each point in the graph

We have attempted to investigate these points amongst patients that ceased to attend or were discharged cured from the Kala-azar Out-patient Department of the Calcutta School of Tropical Medicine during 1924. The routine treatment consisted of injections of a 2 per cent solution of sodium antimony tartrate twice weekly, the dose being increased gradually from 1 cc to 5 cc, or to the limit of the patient's tolerance if this were less than 5 cc.

For each patient who is placed under treatment a card is provided. This card is kept by us, on it the patient's name and address is entered and details of treatment are recorded at each attendance. During the year well over a thousand patients attended for diagnosis but many of these did not remain for treatment. Those patients that had previously received treatment and had relapsed, in the case of whom there was any doubt about the diagnosis, or those that had received less than three injections were not included in this investigation. At the beginning of 1926 a reply postcard was sent to each patient asking for information on certain points with reference to their present condition, as to whether they had received further treatment elsewhere and how many injections they received when attending this institution. We had of course got information on this last point but it was introduced by way of checking the accuracy of the replies and of our records.

The majority of the patients are either illiterate or very poorly educated cultivators, we could not, therefore, expect to receive a very large percentage of replies. About 700 cards were dispatched, 174 satisfactory replies were received, about the same number of cards were returned undelivered by the post office, and the remainder were not returned. Doubtless many of these were lost but probably many of them were received and retained by the patients, either because they were unable to read or write and were not prepared to pay for this to be done for them, or because they were suspicious of the motive and thought that silence was the safer course.

*The accuracy of the information received*—There seems to be every reason to trust the information received as there could be no possible motive in giving inaccurate information. The question with regard to the number of injections was usually answered satisfactorily, and as we had an opportunity of seeing quite a number of the patients we were able to confirm their written statements. Whether the patients that replied were a fair sample is, of course, another matter, but there does not seem to be any reason to suppose that they were not.

No cards were sent out until at least one year after the patient had attended last, so that a patient who replied that he was well and had received no further treatment could almost certainly be considered to have been cured. On the whole it is more probable that information as to the death of the patient was given more readily than any other information, the suspicions as to the motive of the inquiry, referred to above, would not be applicable with reference to a person who was dead. When a patient was reported as dead it was assumed that he had died of the disease, and when he reported that he had received more injections for kala-azar it was assumed that these had been necessary. It is in the last assumption that we are most likely to have gone astray as when a patient has once had kala-azar he imagines that any fever he gets in future is due to



The numbers in these tables have been plotted on graph paper and Figures 1 and 2 drawn. These two figures give a rough graphic representation of the cure rate that may be expected amongst a group of previously untreated patients after courses of treatment consisting of various total doses.

These curves show that the patient who stops treatment even at an early stage in the course of injections has quite a reasonable chance of remaining cured, that a total relative dose of 3 grammes—about 30 injections in an average adult—will effect an 80 per cent cure rate and that prolongation of the treatment beyond this point will only produce a very slight increase in the cure rate.

As there is no evidence, either here or elsewhere, that prolongation of the treatment—up to a reasonable limit of say, 6 grammes—is in any way detrimental to the health of the patient, a very thorough course of treatment should be advocated in the case of the private patient where time and expense are no object, but it is obvious that in dealing with large numbers of patients where the labour involved in giving injections is a matter to be taken into consideration a primary course of not more than 3 grammes per 100 lbs body weight should be advocated and that, where this consideration is of primary importance, a short course of injections given to a large number of patients will produce a greater number of cures than a long course to a smaller number of patients.

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and as the interest does not centre on the actual figures in each group but on the recovery rate, the procedure seemed justifiable

Table II gives the percentage cure rates when the cases are grouped according to the actual total dose given to each patient

TABLE II  
*The actual amount given*

Total dose in grammes	Number of patients	Number cured	Number died	Number relapsed	Percentage cure rate
0.01—0.25	36	8	14	14	22.2
0.26—0.5	21	8	8	5	38.1
0.51—1.0	35	22	7	6	62.8
1.01—1.5	24	17	1	6	70.8
1.51—2.0	30	22	5	3	73.3
2.01—2.5	13	11	2		84.6
2.51 or over	15	13		2	86.7
TOTALS	174	101	37	36	

Again in this table we have not adhered strictly to groups of an equal range. The group of cases receiving less than 0.5 grammes was comparatively large so that it seemed possible to divide it, and all cases receiving more than 2.5 grammes are included in one group as this group was too small to split up.

The usual rule with regard to dosage is that the dose should be in proportion to the weight of the patient. In each instance we have, therefore, calculated the dose per 100 lbs. body weight of patient, this will be referred to as the 'relative' dose. As the weights of the out-patients are not taken as a matter of routine, in order to calculate the total relative dose it was necessary to estimate the patients' weight according to their age. A large number of weights of kala-azar patients have been taken at various times, from these figures two age-weight curves were drawn, one for males and the other for females, and from these curves the weights have been estimated. The average weight of an adult Indian male suffering from kala-azar is 90 lbs., the relative doses have been worked out as the total dose per 100 lbs. body weight of patient, so that the average relative dose is slightly greater than the dose received by an average adult Indian.

The analyses of the two diets are given in Table I

TABLE I

*Showing the analyses of the two diets Results expressed as percentages on moisture-free samples*

Constituents	'Oatmeal Diet' per cent	'Atta Diet' per cent
Mineral Matter	7.20	5.246
Ether Extractives	13.14	9.36
Crude Protein	12.26	12.04
Crude Fibre	1.32	5.09
Carbohydrates	66.08	68.264
TOTAL	100.00	100.00
Albuminoids	11.77	11.41
Insoluble mineral matter	0.465	1.39
Soluble do do	6.735	3.856
TOTAL	7.20	5.246
Soluble Silica	0.0456	0.147
Phosphoric Acid ( $P_2O_5$ )	1.82	1.148
Iron Oxide ( $Fe_2O_3$ )	0.058	0.027
Alumina ( $Al_2O_3$ )	0.462	0.225
Manganese ( $Mn_2O_4$ )	0.0018	0.011
Lime ( $CaO$ )	1.38	0.962
Magnesia ( $MgO$ )	1.32	0.315
Potash ( $K_2O$ )	0.264	0.191
Sulphuric Acid ( $SO_3$ )	0.23	0.084
Chlorine (Cl)	0.49	0.33
Soda and undetermined by difference	0.659	0.416
TOTAL	6.735	3.856

The chemical composition of the two diets differs in certain respects which may have some significance in regard to the greater calculus-producing potency of the 'atta diet'. This diet contains less total mineral matter than the 'oatmeal diet' but more insoluble mineral matter, less phosphorous, iron, alumina, lime, magnesia, potash, soda, sulphates and chlorides, less ether extractives, more crude fibre and more manganese.

The most noticeable points about this table are the low death rate amongst the older children and young adults group and the high death rate amongst adults. The mean actual and relative doses given to the patients in these age groups are given in the following table (Table V)

TABLE V

Age period	CURED		RELAPSED		DIED		ALL PATIENTS	
	Mean actual dose	Mean relative dose	Mean actual dose	Mean relative dose	Mean actual dose	Mean relative dose	Mean actual dose	Mean relative dose
10 years and under 10 years	0.9	2.49	0.60	1.78	0.54	1.35	0.75	2.10
Not over 20 years	1.53	2.29	0.76	1.08	0.67	0.98	1.24	1.84
Over 20 years	1.67	1.85	0.65	0.71	0.81	0.89	1.23	1.35
All patients	1.39	2.21	0.68	1.16	0.72	1.03	1.09	1.74

The mean of the dose given to young children was very low, 0.75 gramme, it is natural that this should be less than the average for all ages as children did not tolerate such large doses, but the dose given in the cases that relapsed was practically the same for all age periods. Furthermore, although the mean of the relative dose given to young children was much greater than the mean of the relative dose given to all patients the cure rate amongst this group was the same as the general cure rate. Again, the mean relative dose of the relapsing cases of the first age group—namely, 1.78 grammes is two and a half times as large as the relative dose of the relapsing cases of the third age group—which is 0.71 gramme—and is practically the same as the mean relative dose of the cured cases of the third age group—namely, 1.85 grammes. These facts suggest that this method of calculating the dosage, directly according to the weight of the patient, is not the method likely to give the most consistent results. Nor on the other hand can the dose be calculated irrespective of the weight and age.

It seems possible that a sliding scale method of calculating might be adopted; as a provisional measure we suggest that more uniform results might be obtained if the mean of the actual and relative dose were calculated. Thus the calculated doses for the three age groups, shown in the above tables, would be 1.42 grammes, 1.54 grammes and 1.29 grammes, these figures are more or less in proportion to the cure rate in these three groups, which was 58, 63 and 51 per cent, respectively.

#### THE CURE RATE CURVE

The figures given in Tables II and III were shown by the senior writer to a medical officer who has had considerable experience in statistical calculations, he expressed the opinion that although curves based on these figures might give some indication of the cure rate for any particular dose the percentages—especially

probable, however, that a number of them died either of pneumonia or of anæmia. In further experiments now in progress the animals have been isolated to avoid cannibalism.

### INCIDENCE OF STONE

The incidence of stone-in-the-bladder was 8 per cent in animals fed on the 'oatmeal diet', 22 per cent in those fed on the 'atta diet' (Tables II and III). The latter diet was, therefore, more potent to cause calculus than the former, this was evidenced not only by the higher incidence of the disease but by the size of the stones and the greater frequency of ureter and kidney involvement. In a previous experiment(1) the incidence of stone was 29 per cent in animals fed on the 'oatmeal diet,' while in the present experiment it was only 8 per cent. The two experiments were carried out in a similar way, but there were the following differences between them. (a) Younger animals were used in the present experiment, their ages at its commencement ranging from 40 to 50 days, whereas in the first experiment the ages of the animals ranged between 60 and 90 days. It seems unlikely that the difference in age could of itself have accounted for the remarkable difference in the incidence and severity of the disease in the two experiments. (b) More minute attention was paid in the present experiments to the hygiene of the animals, but although this may have had some influence in determining the lower incidence of the disease, this influence would not appear to have been great, since the hygienic conditions of the animals in the first experiment were by no means bad. (c) Of more importance, probably, was the season of the year during which the two experiments were carried out. Comparison of the results appears to indicate that in animals fed on the 'oatmeal diet,' stone-in-the-bladder and its sequelæ are more likely to arise during the spring and summer months than during the winter months. This is, however, a tentative conclusion which will require much further work before it can be finally established.

One of the most interesting results of the present experiment is the greater incidence and the greater severity of the disease in the animals fed on the 'atta diet'. Whereas the 'oatmeal diet' gave rise to stone-in-the-bladder in only four cases out of fifty animals, the 'atta diet' gave rise to stone in eleven cases. In three of the former gravel only was present in the bladder and no formed stone while in none was the condition associated with severe cystitis, dilated ureters or diseased kidneys. Four of the eleven cases produced by the 'atta diet' showed gravel only, associated in three with severe cystitis and in one with dilated ureters, hydronephrosis and diseased kidneys. In the 'oatmeal diet' group there was only one case of formed stone, this was comparatively small, weighing 20.6 mgrms. In the 'atta diet' group there were seven cases of formed stones (Plate XXV, fig. 3). The number of the stones varied in individual cases from a single stone weighing 58.6 mgrms (case 36, Table III, Plate XXV, fig. 3) to 20 and 25 weighing 96.4 and 130 mgrms respectively (Plate XXV, fig. 3). It is obvious, therefore, that of the two diets the one containing *atta* (whole wheat flour) was the more calculus-producing—an observation of great interest in view of the extent

might be based on a reasonably large number of observations we have grouped together all the higher doses. The combined table is now as follows —

TABLE VI  
Cure Rate

Dose group grammes	Mean dose of group	Cured	Relapsed or died	Percentage cure rate
<i>Actual dose</i>				
0.01 to 1	0.39	40	61	39.6
1.01 to 2	1.49	47	20	70.1
2.01 over	2.86	84	13	86.6
<i>Relative dose</i>				
0.01 to 1	0.41	21	49	30
1.01 to 2	1.49	31	17	64.6
2.01 to 3	2.44	42	15	73.7
3.01 to 4	3.46	48	9	84.2
4.01 over	5.48	29	4	87.9

Cure rate

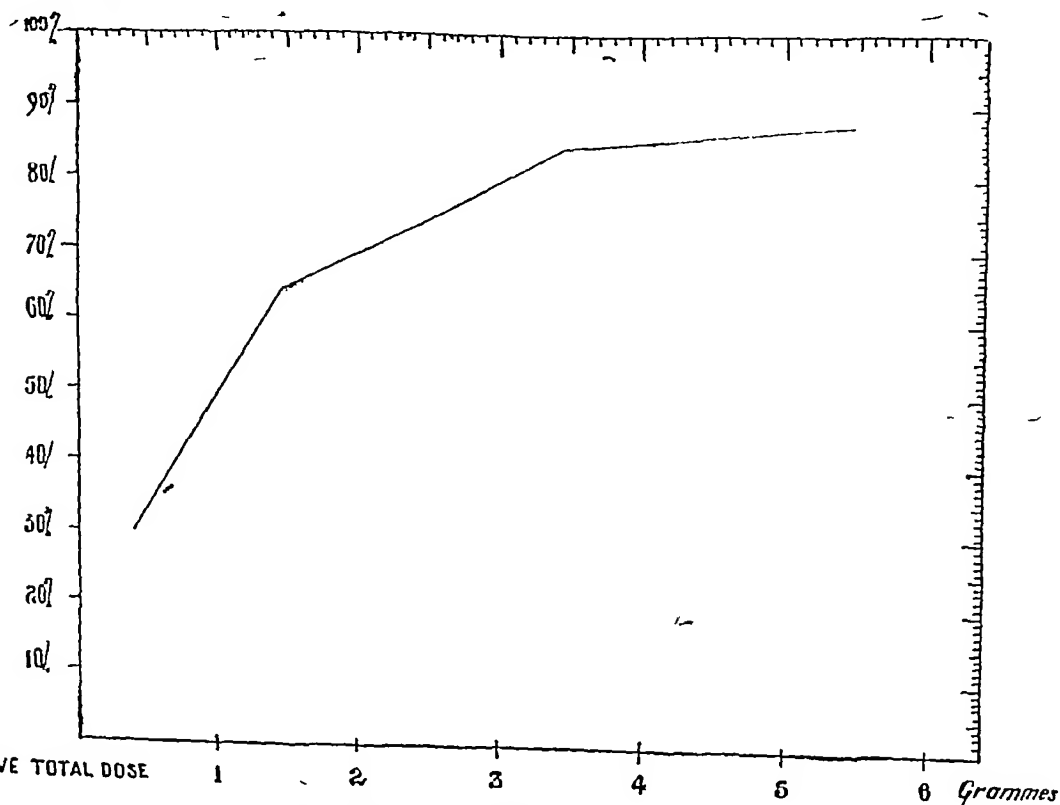


Fig 2—Relative total dose



# THE EXPERIMENTAL PRODUCTION OF STONE-IN-THE-BLADDER

## II.

BY

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IN a previous paper(1) I recorded the experimental production in rats of stone-in-the-bladder, with its sequelæ—cystitis, dilatation of the ureter and hydronephrosis or pyonephrosis—by means of a diet consisting of tinned Scotch oatmeal, linseed meal, cornflour, sodium chloride, calcium phosphate and distilled water. The present paper records the results of a further experiment in which the oatmeal in the above diet was replaced by whole wheat flour (*atta*), the diet so constituted being more calculus-producing than the original 'oatmeal diet'. The composition of the two diets is given below —

### COMPOSITION OF CALCULUS-PRODUCING DIETS

		' Oatmeal Diet '	' Atta Diet '
Oatmeal	per cent	53	—
Whole wheat flour	"	—	53
Linseed meal	"	20	20
Cornflour	"	25	25
Sodium chloride	"	1	1
Calcium phosphate	"	1	1
Distilled water	"	<i>ad lib</i>	<i>ad lib</i>

( 197 )



common      *Dilatation of one or other or both ureters* occurred in five cases out of the fifteen, an incidence of 33·3 per cent. The right ureter was alone affected in one case, the left alone in two cases, and both ureters in the remaining two. *Hydronephrosis* or *pyonephrosis* (the fluid in the dilated hilus of the kidney was not examined bacteriologically, though it was obviously septic in several) was always present in association with dilatation of the corresponding ureter or ureters. In one case a small stone was found in both ureters (No 30, Table III), in another 'gravel' was present in the left ureter. A small stone was found in the kidney in only one case in this series, but one or other or both kidneys were markedly diseased in six cases. The diseased condition of the kidney was apparently due usually to urinary obstruction, but several kidneys had small white miliary areas scattered throughout the cortex and visible at the surface of the organ, one case showed this condition without any associated dilatation of the corresponding ureter (no histo-pathological studies of the diseased kidneys have yet been made). It will be noted from Table III that the sequelæ of calculus were much more frequent in the cases resulting from the 'atta diet'.

The following post-mortem notes, regarding case No 30 (Table III, Plate XXV, figs 1 and 2), will serve to illustrate the appearance seen in a fully developed case. 'The animal is fairly well-nourished but external appearances show it to have been anæmic. On opening the abdomen the bladder is seen to be very engorged and prominent. Both ureters are markedly dilated and are kinked a short distance from their junction with the bladder; the portion between the kink and the bladder is greatly distended giving to the bladder a tri-lobed appearance. The dilatation of the ureters extends up to the kidney on both sides terminating in distension at the hilus of the organ. Pyonephrosis is present on the left side. A very small stone was found in the right kidney. A bunch of stones can be felt jammed into the neck of the bladder (Plate XXV, fig 1). On opening the viscus at this point two large stones one above the other came into view (Plate XXV, fig 2) with two smaller stones on either side of them, the four forming a cross. Much earthy deposit surrounds the two lateral stones, and blocks the orifice of both ureters. On opening the bladder further it is found to be filled with blood-stained mucoid material and to be in a condition of intense cystitis, its walls being greatly thickened and congested. A small stone was present in each ureter lying immediately above the "kink" referred to. On severing the ureters from the bladder and pressing out their contents much earthy matter was removed. This material together with that from the bladder was analyzed quantitatively for oxalates and phosphates. The amount of the former present in the sample was 3·128 mgrms (expressed as calcium oxalate) of the latter 6·134 mgrms (expressed as calcium phosphate).'

#### SEX INCIDENCE OF STONE

In my first series (21 cases) of experimentally-produced stone-in-the-bladder (1) the incidence of the disease differed in the two sexes, being 40 per cent in females and 13 per cent in males. In the present series (15 cases)—taking both diets together—there were 9 cases among 60 females and six among 40 males,

## DETAILS OF THE EXPERIMENT

The experiment was carried out during the winter months, commencing on October 11, 1926, and ending on February 25, 1927 a period of 138 days. It coincided with a spell of unusually dry and cold weather. As will subsequently appear, season of the year seemed to exercise an influence on the incidence of stone.

One hundred young rats were divided into two batches of fifty, and confined in large, roomy cages under conditions of the strictest hygiene. Their age varied from 40 to 50 days. Males and females were equally distributed in the two groups, there being 20 males and 30 females in each. One group received the 'oatmeal diet' in the form of lightly cooked cakes made up with distilled water, the other the 'atta diet' made into similar cakes. The animals were allowed to eat as much as they desired. Distilled water was provided *ad libitum*. The incompleteness of the two diets was made evident by the prevalence of cannibalism. The fate of the animals is shown in Table II.

TABLE II

*Showing the causes of death of 100 rats used in this experiment*

Causes of death	'Oatmeal Diet'	'Atta Diet'
Killed at end of experiment	7	6
Pneumonia (including broncho-pneumonia)	18	12
'Scurvy Œdema'	0	1
Pernicious Anæmia	3	3
Killed(?) and partially eaten by others	12	9
'Waltzing Disease'	1	2
Enteritis	0	1
General wasting (starvation?)	1	0
Abdominal injury	1	0
Unknown causes	3	5
Stone-in-the-bladder and sequelæ	4	11
TOTAL	50	50

Pneumonia was very prevalent owing probably to the relative deficiency of vitamin-A in the two diets combined with the cold weather prevailing during the period of the experiment. It could not be ascertained with certainty whether the 21 animals, noted to have been partially eaten by their fellows, were killed by them or whether they died from natural causes and were partially eaten after death. In the majority of animals dying in this way there was not enough carcass left to enable the point to be decided at post-mortem examination. It seemed

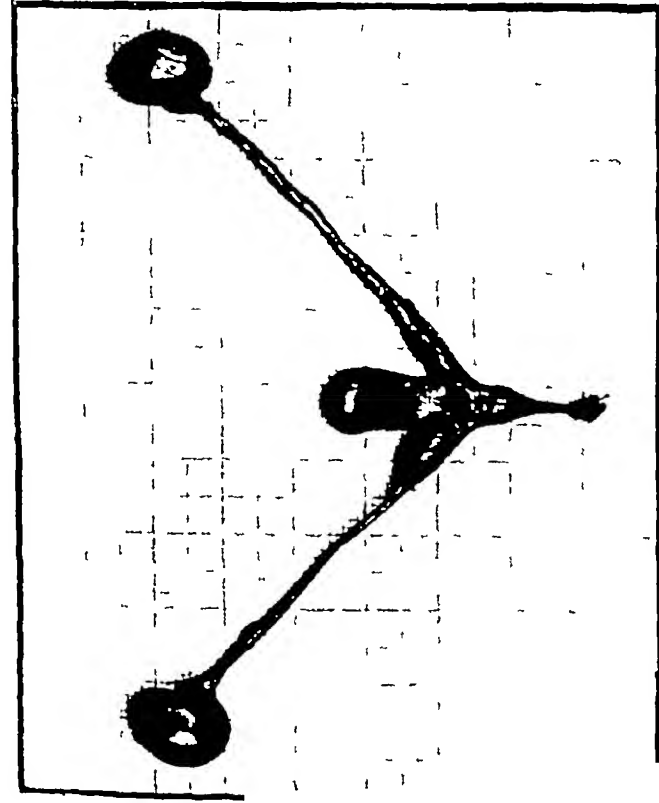
TABLE IV

*Showing the composition of the urine of animals which had received stone-producing diets for 103 to 113 days as compared with that of animals of like age which received a well-balanced vitamin-rich diet*

Volume of urine collected Specific gravity	'Atta Diet.'		'Oatmeal Diet'		Controls A		Controls B	
	94.4 cc		122.2 cc		58.2 cc		64.6 cc	
	1.055		1.048		1.065		1.065	
Material	Total excreted	Per cent	Total excreted	Per cent	Total excreted	Per cent	Total excreted	Per cent
Total mineral matter	3.062	3.40	3.878	3.178	1.688	2.900	2.04	3.14
Total Nitrogen	2.13	2.36	2.15	1.75	1.853	3.914	2.62	4.03
Chlorine (Cl)	1.16	1.28	1.72	1.41	0.077	0.133	0.259	0.398
Sulphates (SO <sub>2</sub> )	0.017	0.019	0.022	0.018	0.014	0.024	0.012	0.018
Phosphates (P <sub>2</sub> O <sub>5</sub> )	0.636	0.706	0.627	0.514	0.766	1.316	0.803	1.236
Lime (CaO)	0.023	0.025	0.046	0.038	0.017	0.028	0.022	0.035
Magnesium (MgO)	trace	trace	trace	trace	trace	trace	trace	trace
Oxalates (Ca C <sub>2</sub> O <sub>4</sub> )	0.039	0.043	0.067	0.055	0.015	0.025	0.016	0.025
Uric Acid	0.101	0.112	0.107	0.088	0.076	0.130	0.083	0.129

#### NOTE ON PREVIOUSLY RECORDED WORK ASSOCIATING STONE-IN-THE-BLADDER WITH FAULTY NUTRITION

In searching the literature for any previous records of the experimental production of stone-in-the-bladder I find in addition to the work of Fujimaki, (2) mentioned in my previous paper, a reference by Osborne and Mendel in 1917 (3) to the incidence of phosphatic urinary calculi in rats fed on experimental rations Padua (1919) (4) also has drawn attention to cystolithiasis among Filipinos in association with dietetic deficiency 'Out of 58 cases of phosphatic calculi, 11 had beri-beri, in 18 cases under-nutrition was observed but without any beri-beri manifestations, 27 cases occurred in well-nourished patients. The phosphatic calculi were found mostly in children and young people, while urate calculi were observed later in life (after 50 years)' (Quoted from Funk) (5). In this connexion it may be mentioned that in India stone-in-the-bladder is less common in Madras—the endemic home of beri-beri—than in the Punjab where beri-beri does not occur. Commenting on these observations Funk (5) remarks that 'a' are associated with the indirect influence of vitamins, presumably decreasing



1—Showing the kidneys, ureters, and bladder from case No 30 (Table III) Fully described in text The kinks in the ureters referred to in text were straightened out when the organs were removed from the body for photographic purposes

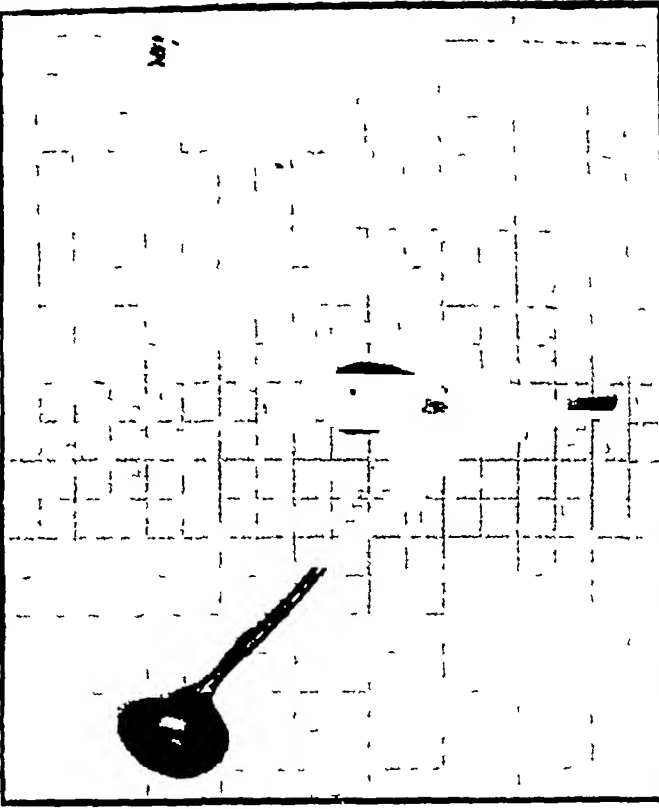


Fig 2—The same neck of the bladder opened showing stones *in situ*



Fig 3 —Showing the calculi present in the bladder in the eight cases having formed stones from the cases are as



TABLE III

Showing the incidence of stone-in-the-bladder and of its sequelæ, with the weight of the calculi, and the sex of the affected animals

Diet	Case Number	Sex	Number of stones	Weight of stones mgrms	Stone in ureter	Stone in kidney	Cystitis	Dilated ureters	Hydro or Pyonephrosis	Diseased kidney.	Manner of death	Days of experiment at death	
Oatmeal, etc	22	F	Gravel	15	no	no	slight	no	no	no	died	68	
" "	23	F	Gravel	—	no	no	no	no	no	no	died	86	
" "	24	F	Gravel	—	no	no	no	no	no	no	died	98	
" "	25	M	1	20.6	no	no	slight	no	no	no	killed	138	Fig 3
Atta, etc.	26	M	4	24	yes	no	no	left	yes	left	died	90	Ditto
" "	27	F	2	35	no	no	severe	right	yes	right	died	90	Ditto
" "	28	M	Gravel	—	no	no	severe	no	no	no	died	100	
" "	29	F	Gravel	—	no	no	severe	no	no	no	died	106	
" "	30	F	5	73	2	yes	severe	both	yes	both	died	109	Figs 1 & 2
" "	31	M	Gravel	11	no	no	severe	both	yes	both	died	110	
" "	32	F	20	130	no	no	slight	left	yes	left	died	136	Fig 3
" "	33	F	25	96.4	no	no	slight	no	no	no	killed	138	Ditto
" "	34	M	Gravel	10	no	no	no	no	no	no	killed	138	
" "	35	M	4	79	no	no	slight	no	no	left	killed	138	Ditto
" "	36	F	1	58.6	no	no	no	no	no	no	killed	138	Ditto

to which *atta* enters into the dietaries of the inhabitants of the 'stone-areas' of Northern India

#### INCIDENCE OF THE SEQUELÆ OF STONE

In my first paper on this subject I drew attention to the fact that in several cases of experimentally-produced stone-in-the-bladder *cystitis* was not present. This point has been carefully noted upon in the present series: *cystitis* in greater or lesser degree was present in 10 out of the 15 cases, little or no *cystitis* was present in the remaining five. Inflammation of the bladder does not, therefore, appear to be a necessary preliminary to the development of stone-in-the-bladder in rats. Although no appreciable *cystitis* may be present, a condition of hypertrophy of the bladder or thickening of its walls has sometimes been noted. Earthy incrustations around the neck of the bladder or at the lower part of its posterior wall, or around the orifices of the ureters have been comparatively

the same disease which I have produced under experimental conditions in man by the administration of the *unboiled* suspended matter present in goitre-producing waters(1), and in rats(2) and goats(3) by the administration of cultures of faecal bacteria, these organisms causing in the experimental animals the various manifestations of the endemic as seen in nature parenchymatous goitre in adults, and congenital goitre, cretinism and parathyroid disease in their offspring. It is the same disease which has been produced by Gaylord(4) in dogs by the administration of the *unboiled* scrapings from the wooden tanks in which goitrous trout were confined.

Pathologically it is a true hypertrophy(17). Overstrain leads to the ultimate development of a diffuse fibrosis with the appearance in the organ of circumscribed areas of solid glandular tissue (*adenoma*) or of colloid-containing vesicles (*colloid adenoma*). The amount of colloid material in the goitre appears to depend in great measure on locality: the further the place of residence of the *goitrous subject from the endemic focus of the disease the greater is the tendency* for the affected gland to store colloid material, the nearer the place of residence to the endemic focus the less colloid the goitre contains and the more it conforms to the parenchymatous or hyperplastic type (de Quervain)(6). In this state of chronic hypertrophy the thyroid is constantly straining to satisfy the needs of the body for its products but just falling short of doing so, consequently evidences of *hypothyroidism* are almost invariably present in older subjects in goitrous regions of Himalayan India, *hyperthyroidism* (thyrotoxicosis) rarely or never. These pathological changes are similar to those occurring in experimentally-produced goitre in animals and in trout confined in tanks(7). In the pregnant female they may result—especially when an additional burden is imposed on the resources of the thyroid gland—in the failure of the maternal thyroid adequately to satisfy the needs of her own tissues and of the developing foetus for the gland's products, with the result that the foetal organ is unduly stimulated and congenital goitre or cretinism may result(8)—a sequence of events which is equally true of experimental animals and of human beings in goitrous zones(9).

This type of endemic goitre possesses distinctive epidemiological characters(10): it has its home in the high mountain ranges of the world, it prevails with different degrees of intensity in different regions and in different parts of the same region, as in villages situated adjacent to one another, the intensity of the endemic is subject to fluctuation, being greater at one time than at another, it has made its appearance in many places where formerly it was unknown, and has disappeared from others, or persisted in them only as isolated cases, it is especially prevalent in agricultural and pastoral districts, though in many towns in endemic zones it may be rife, it is commoner among the poorer than among the richer classes, and in agricultural labourers than in persons of other occupation, many persons may escape it altogether even in regions of high endemicity, it exhibits a distinct seasonal incidence, being more prone to make its first appearance in the spring months in persons not previously affected, or to undergo further enlargement at this season in persons who already suffer from it, epidemics may occur in susceptible individuals or in newcomers to the endemic zone, as in school-children or troops, there is a marked family predisposition

an incidence of 15 per cent in both sexes. The cases occurring as a result of the 'oatmeal diet' are in the present series too few to enable much significance to be attached to the difference in sex-incidence, nevertheless more females than males were affected.

Eleven animals out of 50 fed on the 'atta diet' developed stone-in-the-bladder: 6 among 30 females and 5 among 20 males, an incidence of 20 per cent in the former and 25 per cent in the latter.

#### TIME OF ONSET OF STONE

Without killing a large number of animals at stated intervals during the course of an experiment such as the present one, it is not possible to determine precisely when stone-in-the-bladder first begins to make its appearance. In the first series (1) stone was found only in animals dying between the eighty-fourth and the one-hundred-and-fifty-seventh day of the experiment, in this series 'gravel' was found in the bladder in one animal (fed on the 'oatmeal diet') as early as the sixty-eighth day. The first cases of stone in animals fed on the 'atta diet' were encountered on the ninetyeth day (Nos 26 and 27, Table III), the last in animals killed on the last day (138th) of the experiment. It would appear, therefore, that a period of about 60 days' consumption of the faulty diet is necessary for the production of calculus.

#### URINE ANALYSES

Between the 103rd day and the 113th day of this experiment, the night urine of six animals receiving the 'oatmeal diet' and of six receiving the 'atta diet' was collected in specially constructed receptacles designed to prevent admixture of the urine with faecal material or food. The urine of two groups of six control, well-fed, rats of like age was similarly collected over the same period. A crystal of thymol was added to the collecting bottles to prevent decomposition of the urine. After ten nights a sufficient amount of urine had accumulated for quantitative analysis. The results of this analysis are given in Table IV.

Using the analyses of the urine in the two control groups, and the differences between them, as control figures, the following results emerge in regard to the urine of animals receiving the stone-producing diets —

- (1) The animals receiving these diets seemed to excrete more urine (which is of lower specific gravity) more chlorides, more oxalates, less phosphates and less total nitrogen than control animals receiving a well-balanced food. (Presumably phosphates are precipitated in the bladder and deposited around the forming stones.)
- (2) The animals receiving the 'oatmeal diet' seemed to excrete slightly less mineral matter, less phosphates, less total nitrogen, and slightly more chlorine, lime and oxalates than those receiving the 'atta diet' (Compare composition of the two diets in Table I).



In such sharp contrast are the epidemiological and pathological differences, in these two types of endemic goitre that differences in their etiology can hardly be doubted. It is possible that both may have a common fundamental cause, but if this be so then there are other factors peculiar to certain localities,—such as conditions of hygiene, quality and composition of the food, altitude, soil-composition, hardness of drinking-water, etc.—determining the direction which the initial pathological process shall take whether towards the undue accumulation of colloid material in the organ or the undue proliferation of its parenchyma elements. What these factors may be are unknown at the present time, so far as the genesis of diffuse colloid goitre is concerned. The only information which my own experiments have yielded is that the accumulation of colloid material in the gland appears to be related in some way to disturbance of the 'calcium iodine-balance' of the individual (19).

To these two types of endemic goitre there are probably to be added others of more or less wide distribution. One such may result in rats from the use of food containing more than 60 per cent of white flour and from which green vegetable foods and fruit are excluded. The experimental production of this type of goitre in rats is dealt with in another paper published in this issue (page 247), it may be predicted in regard to it that it will be found to occur sporadically amongst people into whose diet white flour enters largely and green vegetable foods sparingly. Its occurrence is independent of the iodine ingested.

Both types of endemic goitre may occur in the same locality, and intermingled with them may be other goitres unrelated in their origin to endemic influences. Indeed, it is becoming more difficult year by year to find localities in which one type exists alone and in the pure state. Even in the Alps—a home of the classical type of endemic goitre—conditions of improved hygiene, the introduction of protected water-supplies, and changes in the quality of food, must imperceptibly be bringing about alterations in the character, incidence and sequelæ of goitre. It is only in such localities as Himalayan India and the more remote villages of other mountainous regions, where old habits and customs still prevail, and to which the amenities of modern civilization have not yet penetrated, that the classical type of endemic goitre is likely to be still encountered in a state of etiological purity.

During the past twenty-five years I have devoted much attention to the relationship between this type of endemic goitre and unhygienic conditions of life—insanitary surroundings, polluted water-supplies and invasion of the gastrointestinal tract by bacterial agents or their products. The purpose of the investigation with which the present paper deals was to supplement the results previously arrived at and to determine, if possible, to what extent this type of endemic goitre is related to the iodine-content of the soils and water-supplies of regions (Chitral and Gilgit) where the endemic exists in a state of comparative purity.

Samples of soil, of water-concentrates—prepared by evaporation of waters after the addition to them of sodium carbonate—of 'salt,' and of substitutes for 'salt,' were collected from places where this type of endemic goitre raged with great intensity and where its congenital manifestations were abundant, others from villages, some in the heart of an endemic zone, where goitre did not occur.

the resistance to infections, in which connexion vitamin-A is of a special significance. For it is apparent that a local infection is the real cause of this calculus formation. Examples have, however, been given in this paper in which no cystitis was found to accompany the stone. The investigation is still in too early a stage to enable a final decision to be reached on this important point.

#### SUMMARY

(1) The results recorded in my first paper on this subject have been confirmed.

(2) A diet consisting of whole wheat flour, linseed meal, cornflour, salt and calcium phosphate, has been shown to be very potent in causing stone-in-the-bladder and its sequelæ.

(3) The main faults of this diet are its high content of cereal foods and phosphates and its deficiency in vitamin-A, in these respects it approaches in quality that used by the inhabitants of stone-areas in Northern India.

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dissolved in the solutions liberates it slowly. Any method involving titration with thiosulphate is open to the same objection, as to titrate small quantities of iodine with any approach to accuracy, using starch as an indicator, an excess of KI in acid solution must be present. This method proved unsatisfactory in my hands.

The next step in the investigation was to devise a satisfactory means of estimating small quantities of KI (up to 1 mg in 20 c.c.) in pure solution. Some experiments were made with Fresenius' method using chloroform as the liquid for extraction and titrating the iodine with thiosulphate. It was found that the  $\text{HNO}_2$  used for liberating the iodine clung to the chloroform in spite of washing and liberated more iodine from the KI which had to be added before titration. Urea was tried as a means of getting rid of the excess of  $\text{HNO}_2$  but was not satisfactory.

In view of these failures, but more in view of the objections to any method involving titration with thiosulphate and starch, a colorimetric method was tried. Suitable amounts of KI (up to 0.5 mg) were made up in 50 ccm separating funnels to some definite bulk (10, 15 or 20 ccm) with water and sulphuric acid so that the final acidity was about 2.5N. The iodine was liberated and extracted with 10 ccm. of chloroform or carbon tetrachloride. The colour of the iodine in solution was then read against a standard made by treating a suitable amount of N/1000 iodine in the same way. In this method, if the reagents are free from iodine, no iodine is added, so that any found must come from the substance experimented on. There are, moreover, no substances likely to be met with, which give purple solutions in chloroform or carbon tetrachloride except iodine. It was found in some preliminary experiments (1) that about 0.025 mg of iodine in 10 ccm chloroform gave a perceptible colour, (2) that the colour of dilute iodine solutions was approximately proportional to the concentration, and (3) that the presence of  $\text{Na}_2\text{SO}_4$  and some other salts did not affect the liberation or extraction of the iodine, so that the method seemed hopeful.

For sometime, however, the method gave what seemed to be capricious results. On some days the amount of iodine found was from 95 per cent to 100 per cent of the theoretical, but on others only 60 per cent to 80 per cent or occasionally even less. Some experiments on the various agents for liberating the iodine seemed to show that nitrosyl-sulphuric acid ('nitrose') was the best ( $\text{HNO}_3 + \text{H}_2\text{SO}_4$ ) and ( $\text{KNO}_2 + \text{H}_2\text{SO}_4$ ) were also quite effective but the latter gave cloudy chloroform solutions. The standard seemed satisfactory. The N/1000 was prepared by dilution from N/10 made by direct weighing and standardized against bichromate every week or so. Its correction factor was always between 0.992 and 0.993 and this was allowed for. The diluted solution (N/1000) was found to keep for two days unaltered. The chloroform solution does not fade measurably in three hours, evaporation in fact tends to make it stronger but this can be prevented by keeping a layer of water on top. The KI solution was made by direct weighing and gave identical results with another solution made by my assistant, i.e., on the days when the method was giving good results each solution gave from 95 per cent to 100 per cent of theoretical.

# THE RELATION OF ENDEMIC GOITRE TO THE IODINE-CONTENT OF SOIL AND DRINKING- WATER

BY

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## PART I.

### INTRODUCTION

(By R McCarrison)

THE investigations recorded in this paper relate to one type of Endemic Goitre only that variously called 'parenchymatous goitre' in young subjects, 'adeno-parenchymatous goitre' in older subjects, 'chronic hypertrophic goitre' and 'hyperplastic (non-toxic) goitre', the last usually in reference to the experimentally-produced disease in animals. It is the classical type of endemic goitre described by the older writers (Fodéré, Saint-Lager, Baillarger, Kocher, Bircher and many others) as occurring in the Alps and other mountainous regions in association with cretinism, deaf-mutism and idiocy, and is the type which I have studied in Chitral, Gilgit, and other parts of Himalayan India. It is, too, the type of goitre which may occur in animals living in captivity under unhygienic conditions of life, as in artificially-bred trout confined in tanks. It is, I believe,

distinctly acid to litmus, and filtered from a precipitate which forms at this stage, if not before, into a separating funnel and the precipitate washed till the filtrate and washings make up about 70 ccm. The colour of this extract varies from pale yellow to a brownish yellow. Some (generally 1 ccm) N/10 arsenious oxide is then added and left for at least half-an-hour. Two ccm of carbon disulphide are run in and ten drops of nitrore added and the mixture well shaken. The iodine liberated by the nitrore collects in the carbon disulphide phase. In

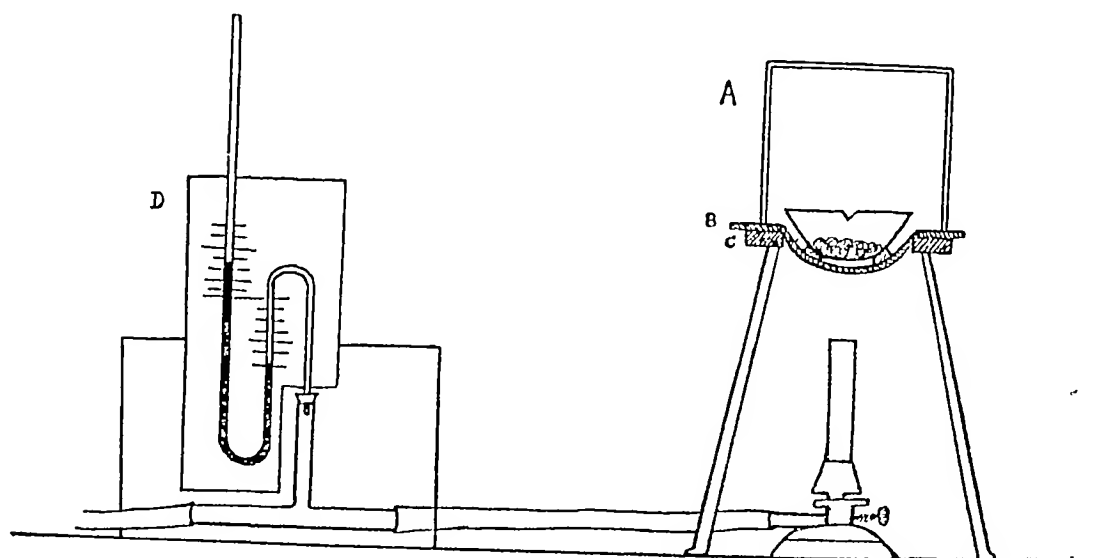


Fig. 1

- A Iron cylinder with open ends 13 cm diam 8 cm. high  
 B Coarse iron gauze impregnated with asbestos and shaped to fit basin. All the gauze inside the ring of the retort stand is impregnated with asbestos  
 C Circular iron ring of the retort stand shown in section  
 D Gauge for measuring the pressure of the gas. The pressure was kept at 5 cm. water  
 The basin is supported on three small pieces of asbestos so that it is about 1 mm. above the asbestos gauze. The temperature on the top of the asbestos gauze will just melt zinc (m.p. 419°C).

some cases the separation of the two phases is not good and the carbon disulphide is mixed up with a brownish scum which prevents the drops from running together. Generally, at least 1 ccm can be got clear by opening the tap of the funnel slowly, but when this is not possible the carbon disulphide can always be cleared by running it and some of the water phase up to a total of about 10 ccm into a centrifuge tube and spinning it at 2,000 revolutions for a few minutes.

To estimate the amount of iodine as much of the carbon disulphide phase as can be got clear is run into a small test tube, graduated into 1/10th of a ccm, and the colour compared with that of a standard solution of iodine in carbon disulphide, the latter is prepared by adding a measured amount of standard potassium iodide solution to 70 ccm of acidified water and arsenious oxide solution in a similar separating funnel and liberating the iodine by the addition of 2 ccm of carbon disulphide and 10 drops of nitrore. As much of the standard as can be got clear is then run into an exactly similar test tube and either the standard or the sample, according to which is darker, diluted with more carbon

or hereditary tendency to the disease, consanguinity favours its development, it is associated with congenital goitre, cretinism (myxoedematous and tetanic)(8), deaf-mutism and idiocy, these sequelæ being as much a part of the endemic as the goitre itself, apart from congenital goitre the malady is comparatively rare in childhood, but it gradually increases in incidence as age advances, attaining its acme between the ages of 50 and 60 years, in regions of low endemicity it is much commoner in females than in males, while in regions of high endemicity the disproportion in the two sexes disappears, there is a marked tendency to spontaneous disappearance of early cases following their removal from the endemic zone, in the endemic zone itself approximately 10 per cent of 'first attacks' of goitre recover spontaneously. The endemicity of this form of goitre cannot be gauged alone by its prevalence in childhood. A proper index is only afforded by an estimate of (1) the number of goitrous subjects in the general population, (2) the proportion of males to females affected, (3) the number of cretinous children, deaf-mutes and idiots, (4) the number of susceptible individuals who acquire the disease within a fixed time, and (5) the size and character of individual goitres. Nothing has done more to confuse the issues involved in the etiology of the 'goitres' than the failure to recognize these criteria on which the endemicity of this disease can alone be based.

This type of endemic goitre is commonly related in its origin to unprotected and impure water supplies, it can be prevented by the substitution of protected and bacteriologically pure water for unprotected and bacteriologically impure water, and it has disappeared from certain places as a result of this measure(10) (11). It can be cured in its early stages by the administration of thyroid substance and of thymol and other intestinal antiseptics(12), by means of vaccines(13) prepared from bacteria of various kinds, and by the internal use of iodine or by its external application, in its later stages it is resistant to iodine-therapy which may then do more harm than good. In whatever way the goitre is caused to disappear the symptoms of *hypothyroidism* accompanying it also disappear(5).

In most of these respects this form of endemic goitre is in marked contrast to the *diffuse colloid type of endemic goitre*. The latter is more especially a disease of the plains and of sea-level regions, most prevalent in children, attaining its acme before and at the age of puberty, and disappearing but for residual changes in the gland, after the age of 25 years (Plummer(14), Levin(15), Olin(16)). It does not appear to be related in its origin to impure water, but there is evidence to suggest that the inadequate ingestion or assimilation of iodine is concerned in its genesis, in its fully developed form it represents an interruption or imbalance of thyroid function (Williamson and Pearse(17)). It is commonly associated in the same locality with Graves's Disease, but there seems to be little statistical evidence in regard to the extent of its association with congenital goitre, cretinism, deaf-mutism or idiocy—such evidence as there is suggesting that these conditions are not an essential feature of the endemic. Pathologically, it is characterized by intense engorgement of the superficial vessels, and by the storage in the gland of colloid material to the almost complete exclusion of active secretion (Williamson and Pearse(17), Plummer(18)).

## THE EFFECT OF CLARIFICATION WITH ANIMAL CHARCOAL

Clarification does not seem to cause any loss of iodine Compare the following experiments —

Soil	Iodine added mgs	Potash added ccm	Heating in minutes	Clarifica- tion	Second heating	Amount of iodine found
M C C	0.3	20	30	Yes	Yes	0.38
M C C	0.3	20	30	No	Yes	0.39

and also the following direct experiment —

A	50 c.c. water + 0.1 mg KI + $H_2SO_4$	
B	Do	Do + animal charcoal
C	Do	Do + KOH + animal charcoal

Each filtered, and C after filtration made acid with  $H_2SO_4$ . To each 1 ccm  $CS_2$  and 8 drops of nitrore. A and C pink and about equal. B colourless.

Showing that KI is adsorbed by animal charcoal in acid medium but not in alkaline.

## THE EFFECT OF A SECOND HEATING

A second heating seems liable to cause a loss of iodine. In two experiments 0.3 mg of iodine as KI plus 10 ccm KOH were evaporated on a sand bath and heated for 15 minutes, and then made acid, filtered,  $As_2O_3$ ,  $CS_2$  and nitrore added. Read against 0.3 mg standard. 0.21 and 0.19 mgs were found indicating a loss of about 33 per cent.

A similar loss is shown in two experiments in which M C C soil with 0.3 mg of added iodine was extracted with water and the iodine estimated in the residue and filtrate separately. In the one case both the residue and filtrate were heated a second time and in the other this second heating was omitted. With the second heating a total of 0.20 mgs iodine was recovered and without it a total of 0.30 mgs.

In the later experiments the second heating was abandoned.

## THE EFFICIENCY OF THE METHOD OF ESTIMATING IODINE IN THE ACID FILTRATE

With pure KI solutions the method is excellent using  $CCl_4$  as the immiscible solvent (*vide ante*). With  $CS_2$  the solution of iodine does not fade if left standing under the water phase and the results would be presumably similar.

Mg,  $(NH_4)$ , Ca, Na, and K ions are without effect on the method but Cl ions, if present in large quantity, may affect it seriously. Up to 25 mgs chlorine ion in 20 ccm is without appreciable effect. With more than this some effect is noticed especially if a large quantity of nitrore is added. This is probably due to the liberation of chlorine. The inhibitory effect of chlorine ions can be avoided if  $As_2O_3$  is used. Using 10 ccm of N/10  $As_2O_3$  solution in 70 ccm water phase, half saturation with sodium chloride is without effect on the liberation of iodine. This proportion of  $As_2O_3$  does not prevent any iodine being liberated. One ccm of  $As_2O_3$  will quantitatively reduce 0.3 mgs of iodate to iodide. Little evidence of any of the iodine being in the form of iodate when extracted from the soil by the ordinary process could be got, but  $As_2O_3$  was added as a precaution, as nitrore liberates no iodine from iodate.

and others, again, from villages where the incidence of the disease was comparatively slight. Similarly, samples of soil were collected from various parts of the Madras Presidency—sea-coast, montane and sub-montane—where endemic goitre is unknown. These samples were numbered and sent to my colleagues who undertook, independently of each other, the estimation of their iodine-content, the sources of the samples being unknown to them. Their investigations involved the preliminary examination of the published methods of iodine-estimation in soil and water, and the evolution by each of a method which appeared to him satisfactory, or as satisfactory as was possible without much further study of the chemical problems involved. Examinations of food for iodine were not undertaken, since in the majority of places from which the soils and water-concentrates were obtained the people are dependent entirely on their own produce in which the iodine-content of the soil, and of the water irrigating that soil, is probably reflected. It is, however, by no means certain that the amount of iodine in the soil bears a very close relationship to the amount of iodine in the vegetable foods grown upon it. It seems not improbable that plants generally, or at any rate some species of plants, may have the capacity of selectively absorbing iodine out of soils. But the complication of imported food-supplies, which might supplement the amount of iodine available in local products, did not concern us in the present inquiry. The only imported food materials were 'rock salt,' and various kinds of 'salty earth' (*numkki nutti*), these were examined separately.

## PART II.

### METHODS OF IODINE DETERMINATION IN SOIL, SALT AND WATER-CONCENTRATES

#### FIRST METHOD

(By *C Newcomb* )

The method of Grutznér(35) (slightly modified for soil) was first tried on a sample of soil from the Madras Medical College compound. The method consists in heating the soil with NaOH and BaO<sub>2</sub>, oxidising the iodine to NaIO<sub>3</sub> with permanganate of potash in alkaline solution, getting rid of the excess of BaO<sub>2</sub> with H<sub>2</sub>SO<sub>4</sub>, and of the excess of permanganate with alcohol in alkaline solution—the hydrated MnO<sub>2</sub> which is precipitated being filtered off, finally, liberating the iodine from the NaIO<sub>3</sub> by the addition of excess of KI in acid solution and titrating it with thiosulphate. Theoretically by this method one gets six atoms of iodine to titrate for every atom in the soil. It is open to the objection, however, that one is adding a substance containing iodine (KI) and many other things besides NaIO<sub>3</sub> liberate iodine from KI in acid solution. Many substances are added and others may have been present originally in the soil which if imperfectly removed might liberate iodine, and even the oxygen



Soil VII with 0 mg iodine added found 0.13

Soil VII with 0.3 mg iodine added found 0.35

Hence the recovery is 74 per cent and this soil contains 0.18 mg/40 grms

Average recovery 68 per cent

### ANALYSIS OF McCARRISON'S SAMPLES OF SOIL

In each case 40 grms of soil were taken

Soil	Iodine added mgs	Potash added cc	Heating in minutes	Clarification	Second heating	Iodine found mg/40g	Figure taken	Parts per 10 millions
I	—	60	60	No	No	1.02	1.29	323
I	—	60	60	Yes	No	1.30		
I	0.3	60	60	Yes	No	1.85		

The last of these three estimations as made on washed soil the iodine being estimated in the residue and filtrate separately

The figure '1.85' is thought to be too high

II	—	60	60	No	No	0.97	—	243
II	—	60	60	No	No	0.47	—	118

These two estimations are selected from amongst several with these two soils as the most satisfactory in that what is thought to be a sufficiency of potash was used and there was no second heating

IV	—	60	60	Yes	No	0.02	0.03	8.0
IV	0.2	60	60	Yes	No	0.17		
V	—	60	60	Yes	No	0.10	0.14	35
V	0.2	60	60	Yes	No	0.24		
VI	—	60	60	Yes	No	0.03	0.04	10
VI	0.3	60	60	Yes	No	0.18		
VII	—	60	60	Yes	No	0.13	0.18	45
VII	0.3	60	60	Yes	No	0.35		

The 'figure taken' for each of the above four samples was arrived at by increasing the iodine found in the soil in proportion to the percentage of added iodine recovered as determined in the second of the estimations. For example taking sample IV —

If  $x$  be the amount of iodine in 40 grms of the soil, and  $a$  the proportion of the total recovered in each determination we have —

$(x + 0) a = 0.02$ ,  $(x + 0.2) a = 0.17$  and hence  $x = 0.03$ ,  $a = 0.75$ , i.e., 75 per cent of the total iodine is recovered

and on the days when the method was giving low results each solution gave results equally low. The KI solution was, therefore, presumably correct.

The capriciousness of the method was finally tracked down to the fact that iodine when liberated by nitroses and extracted by chloroform fades when kept. The fading is due to some substance formed when the nitrose comes into contact with the chloroform. The standard, to which no nitrose is added, does not fade. It is not possible to prevent this fading—although one may slow it down a little—by washing the chloroform phase, or by destroying the excess of nitrose with urea. If, however, carbon tetrachloride be used instead of chloroform the fading does not take place. In this case it is necessary to use carbon tetrachloride for the standard also as the colour is slightly deeper in this extraction agent.

Using  $\text{CCl}_4$  the method gave excellent results with pure KI solutions. Readings of known KI solutions containing from 0 to 0.5 mgs of iodine in 20 ccm against a standard made from N/10 iodine gave results within 0.005 mg when the standard and the KI solutions were not very different in strength, and to within 0.02 mg when one was about double the other. These differences could be still further reduced by making corrections. Variations of (1) the amount of water phase, (2) the acidity, and (3) the amount of nitrose were found to be without effect for small variations. A little over 0.01 mg could just be detected by the method and anything more than this measured.

#### METHOD OF EXTRACTION AND ESTIMATION OF THE IODINE IN SOIL

The method finally adopted for the extraction of iodine from soil is founded on that used by Hercus, Benson and Carter (20), but with important modifications.

The whole sample as received is finely ground in a mortar—stones and all—till it will go through a fine sieve, and the powder then well mixed. The powdered soil is mixed with a 10 per cent solution of potash in a 10 cm diameter nickel dish and dried either on a water bath or over an open flame. During the drying it is stirred with a spatula especially when it has got to the stage of a thick mud so as to break it up into small lumps. When dry it is again powdered and then heated in a constant heating arrangement which raises the temperature to somewhere about  $400^\circ\text{C}$ , for a definite time. This arrangement is shown in Fig 1. By this means it is hoped that the organic matter of the soil will be destroyed and the iodine converted into iodide or possibly some into iodate. After heating it is cooled and extracted with boiling water till the washings are colourless. This takes about 200 to 300 ccm. The colour of the extract varies according to the soil, the time of heating and the amount of potash used. It is generally a dark brown. The extract is then evaporated on a water bath. In the cases in which the extract is very dark it is generally clarified, after its bulk has been reduced to 100 to 150 ccm, by the addition of some 5 to 10 grms of animal charcoal followed by another filtration and a careful washing of the charcoal. The evaporation is then carried on till the bulk is about 30 ccm or in some cases to dryness. In the latter cases the residue is again heated on a sand bath with or without the addition of more potash until it is of a light brown colour, and then taken up in about 30 ccm of water. The extract is now acidified with 5N-sulphuric acid till

'None' in the last column means less than 5 parts of iodine per ten million parts of soil

#### EFFECT OF STORAGE ON IODINE-CONTENT OF SAMPLES

Fellenberg has found that if potassium iodide be added to soil some of the iodine is given off as vapour, the amount so vapourized varying with different soils. It seemed possible, therefore, that soils may give off some of their iodine on keeping or even pick up some from the air. To test this point the following experiment was carried out: a certain soil was taken which was found to contain 28 parts of iodine per 10 millions in two determinations. After exposure to the air and sunlight for 67 hours 28 parts per 10 millions were still found, but after exposure for 261 hours only 20 parts. Conclusions cannot be drawn from a single experiment, but the result is suggestive and merits further investigation. Another factor which may introduce an error into the results, but of what magnitude it would require much study to determine, is the very variable content of water in a soil.

#### THE ESTIMATION OF IODINE IN SALT

In the case of salt the method adopted was as follows —

Ten grammes of the salt were taken, dissolved in water, and made acid with  $5\text{NH}_2\text{SO}_4$ . The solution was filtered and the insoluble matter washed till the filtrate and washings made up 60 ccm. Ten ccm  $\text{N}/10 \text{As}_2\text{O}_3$  were added and left for half-an-hour, and then one or two ccm of  $\text{CS}_2$  and ten drops of nitrose. The resulting colour was read against a standard prepared by adding 10 grms of salt, free from iodine, to 60 ccm of acidified water and a suitable amount of standard KI solution. The same amounts of  $\text{As}_2\text{O}_3$ ,  $\text{CS}_2$  and nitrose were added to the standard as had been used for the unknown.

With common salt bought in the bazaar this method gave no iodine but would detect 0.02 mg added to 10 grms (2 parts per million). The recovery of more added iodine was by this method 100 per cent within the limits of error of the colorimetric readings.

An attempt was also made to concentrate the iodine in salt by a method of fractional precipitation with alcohol similar to that described in Treadwell and Hall's Analytical Chemistry, 1907, Vol II, p 521. 30 grms of salt were taken to start with and were concentrated to 10 grms or less and then the iodine estimated as above. A certain amount of iodine, however, seemed to be lost in the process and in the most successful experiment with this modification 0.03 mgs in 30 grms (1 part per million) was only just detected.

The method supposes the iodine to be in the form of iodide or iodate. Presumably if the iodine were in some organic or other combination as in soils the method would fail to detect it.

#### ANALYSES OF MCCARRISON'S SALT SAMPLES

Sample	Amount taken	Insoluble matter	Concentrated	Iodine found	Figure taken	Parts per 10 millions
XI	10	1.3%	{ no	0.07	0.07	70
XI	30		{ yes	0.09		
XXIX	10	10.8%	no	none		less than 20
XXX	10	none	no	none		less than 20

disulphide until the colours match From the amount of dilution necessary the amount of iodine is calculated by a simple proportion

In many cases the residue of the water phase is tested for the presence of any substances which might inhibit the liberation of iodine, by the addition of 0.05 to 0.10 mgs iodine in the form of KI, and 1 or 2 ccm of fresh carbon disulphide In nearly every case in which this was tried this added iodine was recovered quantitatively indicating the absence of any such inhibiting substances Where no iodine is found the effect of more  $As_2O_3$  and more nitrose is tried, but in all cases without any alteration in the result

#### THE EFFECT OF THE TIME OF HEATING AND THE AMOUNT OF POTASH

The effect of the time of heating does not appear to be very great when the standard heating arrangement is used, except that the longer the soil is heated the lighter is the colour of the extract If the heating is conducted over an open flame and the dish is allowed to become red hot then iodine appears to be lost The following experiments show this —

Soil	Iodine added mgs	Potash added ccm	Heating in minutes	Clarifica- tion	Second heating	Amount of iodine found
M C C	0.2	20	15	No	No	0.13
M C C	0.2	20	45	No	No	0.14
M C C	0.2	20	60	No	No	0.14
M C C		20	20	No	Yes	0.09
M C C.		20	50	No	Yes	0.07

These results are too low but the effect of the time of heating does not seem to be marked

The amount of potash added is of great importance —

III	—	40	60	No	No	0.23
III	—	60	60	No	No	0.47
III	—	100	60	No	No	none
M C C	0.2	20	60	No	No	0.14
M C C	0.2	92	60	No	No	0.30

Showing that with insufficient potash much iodine is lost

The following experiments show the combined effect of time of heating and the amount of potash —

I	—	20	30	No	Yes	0.23
I	—	60	60	No	No	1.02
II	—	20	30	No	Yes	0.34
II	—	60	60	No	No	0.97
III	—	20	30	No	Yes	0.15
III	—	40	60	No	No	0.23
III	—	60	60	No	No	0.47

## ESTIMATIONS OF IODINE IN WATER CONCENTRATES

The estimation of iodine directly in the concentrate by making it acid, filtering and liberating the iodine from the filtrate, was found unsatisfactory in the one case in which it was tried (XXXV), owing to something in the filtrate interfering with the liberation of the iodine

The following method was therefore adopted —

From 50 to 100 ccm of the concentrate were taken according to the amount available, 10 ccm of potash added and the mixture evaporated. During the later stages of the evaporation 20 grms of sand, free from iodine, were added. When dry the mass was heated on the constant temperature apparatus for 30 minutes, and then treated as for a soil. No clarification was needed as the extracts were all nearly colourless. No second heating was used.

With each of the concentrates two estimations were done, one without any and one with 0.1 mg of iodine in the form of KI added. The recovery of small quantities (0.02 to 0.04) of iodine added to the residue of the water phase after the estimation was always tried and in each case the recovery was good (i.e., over 75 per cent). Using this method it is thought that 0.005 mg of iodine would just be detectable.

Total solids, including sediment, of each of the concentrates were also estimated by evaporating 5 ccm of the well shaken concentrate to constant weight on a water bath.

## ANALYSES OF McCARRISON'S WATER CONCENTRATES

Number	Total vol ccs	Total solids gr/100 ccs	Volume taken ccs	Iodine added mgs	Iodine found mgs	Figure taken	Per cent of added iodine recovered	Amount of iodine in mgs/litre = parts in a million of concentrate
XXXI	300	2.46	100 100	0 0.1	trace } 0.056 }	0.01	51	0.1
XXXII	320	1.46	100 100	0 0.1	0.010 } 0.104 }	0.01	94	0.1
XXXIII	115	7.38	50 50	0 0.1	0.024 } 0.118 }	0.02	94	0.4
XXXIV	220	1.54	60 60	0 0.1	? trace } 0.070 }	? 0.01	65	? 0.2
XXXV	342	1.88	80 80	0 0.1	none } 0.09 }	none	90	none

It is doubtful if XXXIV contains any iodine, but if any, then not more than 0.01 mg in 60 cc = 0.2 parts per million.

No iodine was detected in XXXV and had more than 0.005 mg in 80 cc been present it is thought that it would have been found. 'None' here means less than 0.07 parts per million.

I am indebted to my research assistant Mr B. G. Krishnamurthi for assistance in most of these experiments and to my other research assistant

One drop of nitrose in 20 ccm is enough to liberate over 0.8 mgs iodine, but when  $\text{As}_2\text{O}_3$  is added this proportion is not enough to liberate all the iodine even when a much smaller amount than 0.8 mg is present. Ten drops will liberate all, or very nearly all, the iodine whether 1 ccm or 10 ccm of  $\text{N}/10 \text{As}_2\text{O}_3$  are used. If a lot of  $\text{As}_2\text{O}_3$  is added a large excess of nitrose is to be avoided as it oxidizes some of the liberated iodine further, especially if much chlorine ion is present.

The evolution of  $\text{CO}_2$ , when acid is added to a solution containing a carbonate and KI, does not carry off any iodine. The presence of  $\text{SO}_2$  prevents the liberation of iodine by nitrose.

### REAGENTS

The reagents used were free from appreciable amount of iodine. Numerous experiments in which no iodine was found indicate this. The KOH was specially tested for iodine by making 40 ccm of it acid and adding  $\text{As}_2\text{O}_3$ ,  $\text{CS}_2$  and nitrose. No pink colour resulted indicating less than 0.02 mg iodine in 40 ccm. The nitrose was prepared by passing nitrous fumes, got by the interaction of starch and nitric acid, into a strong sulphuric acid.

The KOH solution was made from sticks 'pure from alcohol' by dissolving 100 grms in a litre of water.

The  $\text{H}_2\text{SO}_4$  used was 5 N.

The  $\text{CS}_2$  was Hopkins and Williams 'A.R.'

The KI solution contained 1 mg iodine per 10 ccm. It was made by weighing out KI and standardized against bichromate.

In all cases 40 grms of soil were taken for each experiment. 'M.C.C.' means Medical College Compound, Madras. It is also called sample VIII.

### RECOVERY OF ADDED IODINE

Most of the experiments on the recovery of added iodine were made before the necessity of adding a sufficiency of potash and the loss on second heating had been discovered. The following experiments however indicate that using 60 ccm potash for 40 grms of soil, heating for 60 minutes and omitting the second heating, the recovery is somewhere about 70 per cent.

Soil IV with 0 mg iodine added found 0.02 mg

Soil IV with 0.2 mg iodine added found 0.17 mg

Hence the recovery is 75 per cent and this soil contains 0.03 mg | 40 grms

Soil V with 0 mg iodine added found 0.10

Soil V with 0.2 mg iodine added found 0.24

Hence the recovery is 70 per cent and this soil contains 0.14 mg | 40 grms

Soil VI with 0 mg iodine added found 0.02

Soil VI with 0.3 mg iodine added found 0.18

Hence the recovery is 53 per cent and the soil contains 0.04 mg | 40 grms

The filtrate is then transferred to a separating funnel and 2 ccm of carbon disulphide added. Ten drops of nitrore (specially prepared from starch and nitric acid) are added and the whole well shaken. Iodine is liberated and is dissolved out by the carbon disulphide. It is allowed to settle.

The top layer is decanted off into another separating funnel so that as little of the carbon disulphide passes over as possible. The iodine is then washed twice with 20 ccm of water each time and similarly decanted into the same separating funnel. About 1 to 2 ccm of saturated sodium acetate solution are added to the carbon disulphide and shaken. The whole is then withdrawn into a stoppered test tube in which the titration of the iodine is conducted. A small quantity of carbon disulphide passes over while decanting and is found collected in the other separating funnel. It is withdrawn from the latter, on to a funnel fitted with a *wetted* filter paper. It is washed twice with a jet of water and then transferred, by puncturing the filter paper, to the stoppered test-tube.

The iodine is then titrated against  $N/1270 \text{ Na}_2\text{S}_2\text{O}_3$ . The disappearance of the colour can be seen to within about 0.1 ccm of the thiosulphate of the above strength. About the same range of sensitiveness as Hercus and his co-workers has been found, i.e., working with 50-gm portions of soil it is possible to detect 2 parts of iodine in 10,000,000 parts of soil.

The method employed in estimating iodine in water concentrates was the same as for soils.

#### THE ESTIMATION OF IODINE IN SALT

In the presence of large quantities of sodium chloride, nitrore failed to liberate the small quantities of iodine that are usually met with in these samples. The method of Kendall was, therefore, adopted to suit the present case. No ignition was conducted. The iodine is oxidized by bromine to iodate, the excess of bromine driven out by boiling and the iodate estimated by determining the iodine it liberates from added potassium iodide.

Duplicate experiments with pure salts were at first conducted. 2-ccm portions of potassium iodide solution, containing about 0.1 to 0.2 mg of iodine, were subjected to the above operations with and without the addition of 10 gms of sodium chloride. The following are the titres obtained —

2 ccm of potassium iodide only	1.5 c.c.
do do	1.45 c.c.
2 ccm of potassium iodide solution plus 10 gms of sodium chloride	1.55 c.c.
do plus do	1.5 c.c.

The agreement is so close as to justify the conclusion that the presence of NaCl is harmless. Sodium chloride alone was tried because the salts when examined qualitatively, gave indications of large quantities of chloride and carbonate and only traces of sulphate.

The results of analysis are given in a separate tabular statement appended. More than one figure in the case of some samples represent repetitions.

Soil	Iodine added mgs	Potash added cc	Heating in minutes	Clarification	Second heating	Iodine found mg/40g.	Figure taken	Parts per 10 million.
VIII	—	20	20	No	Yes	0 09	0 10	25
VIII	—	20	40	Yes	No	0 10		
VIII	—	20	50	No	Yes	0 07		
VIII	0 3	20	30	Yes	Yes	0 38		
VIII	0 3	20	30	No	Yes	0 39		
VIII	0 05	20	30	Yes	Yes	0 14		
VIII	0 05	20	30	No	Yes	0 06		
VIII	0 3	20	30	No	Yes	0 20		
VIII	0 3	20	30	No	No	0 20		
VIII	0 2	20	45	No	No	0 14		
VIII	0 2	20	60	No	No	0 14		
VIII	0 2	20	15	No	No	0 13		
VIII	0 2	92	60	Yes	No	0 30		

In most of these estimations the amount of potash added is not sufficient. In spite of this the first six agree remarkably well amongst themselves. The 'figure taken' is arrived at by a consideration of all the estimations, giving special weight to those in which enough potash was added and the second heating was omitted. This soil is from the Medical College Compound, Madras.

IX	—	60	60	Yes	No	0 08	—	20
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Sample IX is from a dry paddy field in Nungambaukam, Madras, three miles from the sea as the crow flies.

X	—	60	60	No	No	0 15		38
XII	—	60	60	No	No	none	? 0 02	5 0 ?
XII	—	60	60	No	No	? trace		
XIII	—	60	60	No	No	none	? 0 02	5 0 ?
XIII	—	60	60	No	No	? trace		

It is not certain that samples XII and XIII contain any iodine but if any not more than 5 parts per ten millions.

XIV	—	60	60	No	No	0 03	0 06	15
XIV	—	60	60	Yes	No	0 06		
XV	—	60	60	No	No	none		none
XV	—	60	60	No	No	none		
XVI	—	60	60	Yes	No	none		none
XVII	—	60	60	No	No	none		none
XVIII	—	60	60	Yes	No	none		none
XIX	—	60	60	Yes	No	none		none
XX	—	60	60	No	No	none		none
XXI	—	60	60	No	No	none		none
XXII	—	60	60	Yes	No	none		none
XXIII	—	60	60	Yes	No	? trace		? 5 0
XXIV	—	60	60	Yes	No	none		none
XXV	—	60	60	Yes	No	0 03		8 0
XXVI	—	60	60	No	No	? trace		? 5 0
XXVII	—	60	60	Yes	No	none		none
XXVIII	—	60	60	No	No	0 08		20 0
XXVI	—	60	60	No	No	none		none
XXXVII	—	60	60	Yes	No	0 03		9



*Results of analyses of samples of soils, salt and water concentrates for iodine-content, received from Lt-Col R McCarrison—contd*

Number	Description	Iodine content parts per 10 000,000
XXV	Soil	4
XXVI	do	6
XXVII	do	less than 2 parts
XXVIII	'Nimiki mitti'	18
XXIX	Salt powdered by boiling a certain earth with water *	traces
XXX	Rock salt *	do
XXXI	Water concentrates	do
XXXII	do do	nil
XXXVI	Soil	less than 2 parts
XXXVII	do	" " 5 "
XXXVIII	do	" " 4
XXXIX	do	" " 3 "
XL	do	" " 3 "
XLI	'Nimiki mitti'	" , 5 "

\* These samples were analysed by a modification of Kendall's method (21) Sample XI contained some organic matter which was oxidized by bromine but on cooling a white cloudy precipitate settled which had to be filtered off

### THIRD METHOD

(By R V Norris)

The estimation of iodine in organic matter and soils present considerable difficulties as, the amount present being as a rule very small, large quantities of material have to be employed The destruction of the organic matter is essential, and to do this effectively without loss of iodine is one of the main difficulties

Sample XXIX in view of the large amount of insoluble matter was also treated as a soil 30.8 grms were taken and evaporated and heated with 54 ccms potash for 30 minutes, and then extracted as usual In view of the large amount of salt present only an aliquot part of the extract was taken for the liberation of iodine in one case 30|100 in another 50|100 In neither case was any iodine found and the recovery of iodine added to the residue of the extract was good It is thought that 1 part in a million would have been detected by this method

#### EXPERIMENTS ON THE WASHING OUT OF IODINE FROM SOIL

Several experiments on washing soil with cold (Temp c 34 C) water were made and estimating the iodine in the residue and filtrate separately In some of them an insufficiency of potash was used and the yields are low, but they all indicate that the iodine present naturally in the soil cannot easily be washed out while iodine added to the soil in the form of KI can be washed out readily

In each case 40 grms of soil were taken and washed with about 250 ccm water and the iodine estimated in both the residual soil and in the water filtered off from it

Soil	Iodine added mgs	Iodine found		
		in filtrate	in residue	Total
I	0	0.03	1.27	1.30
I	0.3	0.30	1.55	1.85

Sixty ccm potash and 60 minutes heating, and no second heating were used in the above The figure 1.55 is perhaps too high

Soil	Iodine added mgs	Iodine found		
		in filtrate	in residue	Total
M C C	0	0	0.04	0.04
M C C	0.3	0.18	0.02	0.20
M C C	0.3	0.25	0.05	0.30

Twenty ccm potash and 30 minutes heating in these three The first two were heated a second time but the last not From other experiments M C C soil is thought to contain 0.10 mg iodine per 40 grms soil The results are all low but they indicate that the same as the experiments with soil I

The apparatus used is shown in the diagram (Fig 2) which is self-explanatory. The combustion was continued for about one hour by which time the residue is quite free from iodine. The alkaline solution and the washings of the absorption vessel were transferred to a 100 ccm stoppered bottle and carefully neutralized with sulphuric acid. The iodide present was oxidized to free iodine by the addition of a few drops of nitrososulphuric acid and the iodine taken up in 2 ccm of carbon tetrachloride. The carbon tetrachloride solution was separated, washed with a very small quantity of distilled water and any acidity removed by the addition of 2 ccm of a concentrated sodium acetate solution. If considered desirable the extraction can be repeated. The iodine in solution was then determined by titration with N/1270 sodium thiosulphate.

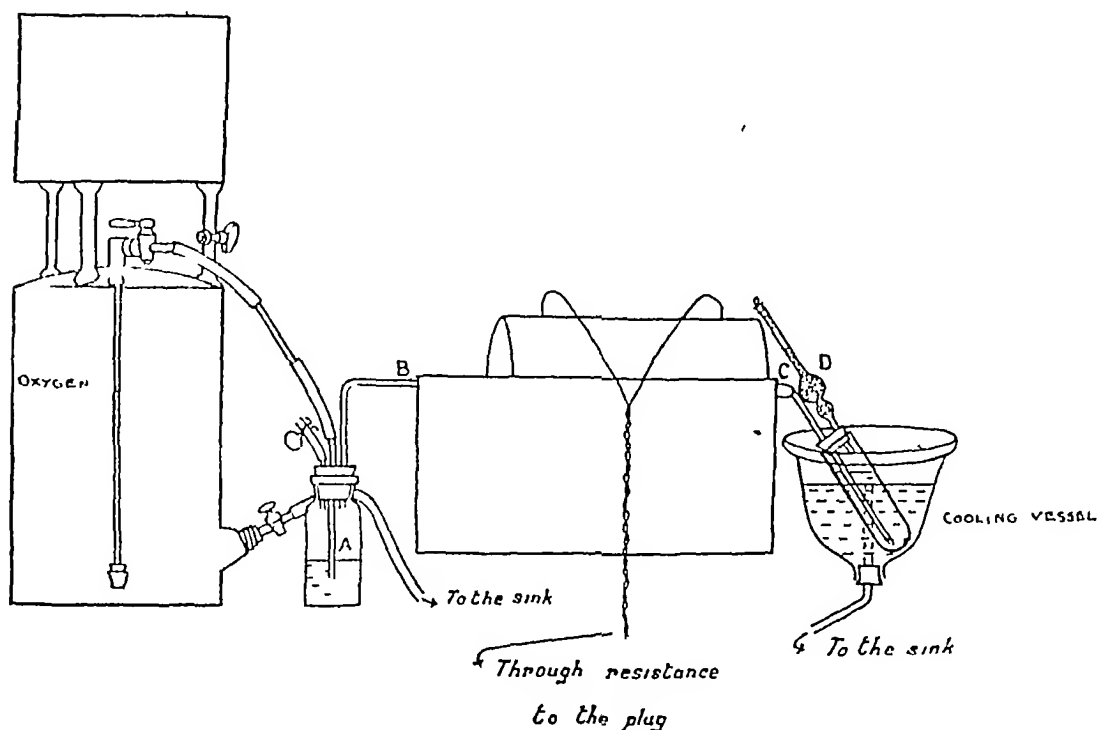


Fig 2

This method was found to give generally satisfactory results and if the iodine present in the soil exceeds about 25 parts per 10 millions the estimation can be carried out on 5 grms of soil. For smaller iodine contents larger quantities of soil should be employed. The method was tested out using soils with known amounts of added iodine.

The results obtained with Col McCarrison's series of Indian soils are shown in table below. In the case of soil containing less than 10 parts per 10 millions the results are simply shown as 'less than ten' as when this series of analyses was made the amount of soil that could be used was limited by the small size of the silica tube available and figures below ten were not, in consequence regarded as significant.

I am indebted to Mr D A Rama Rao, a research student in my laboratory, who carried out the above analyses under my direction.

Mr G Sankaran for his help in some of the latter ones, and to the South Indian Branch of the British Medical Association for a grant from which the salaries of my assistants were paid

## SECOND METHOD

(By *B Viswanath*)

The method followed is essentially that of Hercus and his co-workers(20) and consists of low ignition with alkali until organic matter is destroyed, acidifying, liberating the iodine with 'nitrose,' and estimating it volumetrically

### DETAILS OF THE METHOD

*Preparation of the samples*—The sample of soil is well pounded and passed through 1 mm sieve and bottled up for analysis. 50-gm portions are weighed into a nickel dish and saturated with 8 per cent NaOH (purified by alcohol), dried on a water bath, and powdered fine in a porcelain mortar. It is then re-transferred to the nickel dish and ignited by waving constantly a bunsen flame beneath it so that the dish is always well below dull red heat. (Initial experiments on the temperature of ignition have indicated that at no time should the dish be heated to dull red heat. A sample of soil heated to somewhere between 610°C and 715°C lost all its iodine. On the other hand, even a full red heat is not able to expel the last traces of iodine from a sample. The best temperature of ignition seems to lie somewhere in the vicinity of 400°C. A much lower temperature fails to destroy all organic matter which is essential. This is corroborated by the fact that in Kendall's method the temperature of ignition is that of molten sodium hydroxide, i.e., 318°C.) The ignition takes about half-an-hour. The soil is then extracted with hot water. The extract should be either colourless, or slightly straw-coloured if the ignition has been complete. But more often than not, the extract is coloured deep brown. But it is essential that it should be only slightly coloured if at all. Therefore, the soil is extracted with about 200 to 250 ccm of water, the extract evaporated and heated again in the same manner for another 10 minutes and then proceeded with

(Hercus and his co-workers seem to have generally obtained a clear extract after 15 minutes ignition. In no case, however, did a half-an-hour ignition give us clear extracts. It was only in the case of some sandy soils, that this was realized. In all other cases, the ignition was prolonged and very often had to be repeated on the evaporated extract. In certain soils rich in iodine—as in samples I and II—extraction to the extent of 70 ccm is far too small. This is particularly so if the extract happens to be coloured.)

The ignited residue is taken up with about 30 ccm of water and neutralized with dilute sulphuric acid in an Erlenmeyer flask till it is slightly on the acid side. It is then filtered (the filtrate *should be colourless*, or at the most straw-coloured, otherwise, all the iodine is not liberated by 'nitrose' and the value obtained is too low) and washed until 70 ccm of filtrate are obtained

TABLE I

Showing the results of iodine estimations in Soils and the incidence of goitre

Sample Number	Source of sample	Endemicity of goitre per cent	Iodine in parts per 10 millions			REMARKS
			A	B	C	
I	Coonoor, Nilgiris	nil	323	414	400	First group—Endemic goitre unknown, but in the neighbourhood of Coonoor (samples I, II and III) 'incipient goitre' occurs in school-children to the extent of 24·1 per cent in girls and 11·2 per cent in boys, small goitres to the extent of 3·6 per cent in girls and 0·57 per cent in boys
II	Springfield, Nilgiris	nil	243	259	300	
III	Jungle, Nilgiris	nil	118	101	400	
VIII	Madras city	nil	25			
IX	Nungabaukam, Madras	nil	20			
VIIIa	Coimbatore	nil		23		
IXa	Coimbatore	nil		45		Second group—Localities in or in neighbourhood of, endemic zones, goitre not endemic
XXXVIII	Madras Garden soil (a)	nil		4	less than 10	
XXXIX	Madras Garden soil (b)	nil		3	" "	
XL	Madras Garden soil (c)	nil		3	" "	
VIIIb	Bangalore plateau	nil			" "	
IXb	Bangalore plateau	nil			" "	
VI	Kasauli garden	nil	10	4	" "	
VII	Kasauli hillside	nil	45	32	" "	
XIII	Hunza, Gilgit	nil	less than 5	25	" "	
XIV	Barmis, Gilgit	nil	15	45	" "	

*Results of analyses of samples of soils, salt and water concentrates for iodine-content, received from Lt-Col R McCarrison*

Number	Description	Iodine-content parts per 10,000,000
I	Soil	404 428 412
II	do	262 255 261
III	do	96 101 107
IV	do	40 50
V	do	220 160
VI	do	40 40
VII	do	320 320
VIIIa	Permanent Manurial plots 'No Manure'	230
IXa	do do 'Cattle'	450
X	Clay	280
XI	Salt produced by evaporation of a certain spring water	75
XII	Soil	50 30
XIII	do	20 30
XIV	do	50 40
XV	do	traces
XVI	do	20
XVII	do	less than 2 parts
XVIII	do	100
XIX	do	30
XX	do	30
XXI	do	less than 2 parts
XXII	do	" " 2 "
XXIII	do	" " 2 "
XXIV	do	" " 2 "

TABLE II  
Showing the results of iodine estimations in Salt and substitutes for salt and the incidence of goutre

Sample Number	Source of sample	Endemicity of goutre per cent	Iodine in parts per 10 millions			REMARKS
			A	B	C	
XI	Nat-ye-Chaung spring, Chm Hills by evaporation	45	70	75		The water of this spring has a high local repute for curing goutre
XXVIII	Salt earth (numk: miti) Hunza	nil	20	18	less than 10	Earth used as substitute for salt
XXIX	Hunza salt	nil	less than 2	traces	" " 10	Prepared by boiling a certain earth in water and subsequent evaporation
XXX	Imported rock-salt	11 8 to 45 6	" " 2	traces	" " 10	In use in Gilgit by those who can afford to purchase it
XLI	Salt earth (numk: miti) Nagar	10	6	5		Earth used as a substitute for salt

A Major Newcomb's results, B Mr Viswanath's results, C Dr Norris's results  
Percentages in column 4 represent incidence of goutre in general population Only well-defined goitres included

The usual method employed [Kendall(21), Kelly and Husband(22), Pickworth(23), etc], is to fuse the material with caustic soda, the destruction of organic matter being assisted in some cases by the addition of potassium nitrate or other oxidizing agents. The ignited mass is extracted and after suitable steps to bring all the iodine present in the extract into the form of free iodine the latter is usually taken up in carbon disulphide or carbon tetrachloride and estimated either colorimetrically or by titration against very dilute thiosulphate. McClendon(24) oxidises the organic matter in a somewhat different method, the material being mixed with lime and heated in a combustion tube in a stream of oxygen. The volatile products are absorbed in alkali and worked up together with the washings from the residue in the combustion tube.

The above methods have been applied generally to body fluids, foodstuffs, etc, and but little work is on record relating to the estimation of iodine in soils(25). The difficulty in the latter is the large amount of inert material present which increases the difficulty of destroying the organic matter present while the extraction of the residue left after ignition is also troublesome. The largest series of soil analyses is that given by Hercus, Benson and Carter(20) who give figures obtained with some 400 New Zealand soils. Their method consists in moistening the soil with 8 per cent sodium hydroxide, igniting at a low red heat for 15 minutes and extracting the iodide with boiling water. The extract is neutralised, the iodide oxidized to free iodine by nitro-sulphuric acid, taken up in carbon disulphide and titrated with dilute sodium thiosulphate.

No figures for control soils with known amounts of added iodine are given by Carter in his paper.

In view of the simplicity of Carter's method, it was decided to make a trial of this in connexion with the present series of Indian soils. Many Indian soils, however, contain high percentages of iron and in view of the known influence of iron in causing loss of iodine it was considered advisable in the first place to test out the method with soils rich in iron content and containing known amounts of added iodine.

In such cases very low results were invariably obtained by Carter's method. That this loss was due to the iron content of the soil would seem to be indicated by the fact that quartz sand gave accurate results whereas if the sand had previously been ignited with an iron salt and thus impregnated with oxide of iron the results were again low. It is probable that in such cases there is separation of free iodine at comparatively low temperature and consequent loss by volatilisation. It was therefore decided to carry out the ignition in a combustion tube and to absorb the products in alkali to fix any iodine. To simplify the procedure no alkali was added to the soil, the object being to drive over the whole of the iodine into the absorption vessel and so obviate the necessity for washing the soil residue. Preliminary experiments showed that this could be done with certainty provided the temperature was raised to about 900°C. The combustion was consequently carried out in a nickel boat in a silica tube in a stream of oxygen, the combustion tube being heated in a simple type of electric tube furnace to about 900°C. The gaseous products of the combustion carrying the iodine were absorbed in a tube containing about 15 cc of 15 per cent sodium hydroxide.



## PART III.

## DISCUSSION

(By R McCarrison)

## A THE IODINE-CONTENT OF SOIL IN RELATION TO ENDEMIC GOITRE

The 35 samples of soil (Table I) range themselves into four groups according to the incidence of goitre in the places from which they were derived —

- (a) Those from localities in which endemic goitre is unknown (I, II, III, VIII, IX, VIIIa, IXa, VIIIb, IXb, XXXVIII, XXXIX and XL)
- (b) Those from localities in, or in the neighbourhood of, endemic zones in which endemic goitre did not occur (VI, VII, XIII and XIV)
- (c) Those from localities in the endemic zone in which the incidence of the disease was slight (XXIV and XXV)
- (d) Those from localities in the endemic zone in which goitre and its sequelæ (cretinism, etc.), prevailed with varying degrees of intensity (the remaining samples)

(a) *In the first group* there are three soils (I, II and III), from a mountainous district (Nilgiri Hills), in which the iodine-content is very high, 100 to 400 parts per 10 millions, four (VIII, IX, VIIIa and IXa), from sea-coast and sub-montane localities in which it is moderately high, 23 to 45 parts per 10 millions, and five (XXXVIII, XXXIX, XL, VIIIb and IXb), from a sea-coast district and the Bangalore plateau in which it is very low, less than 10 parts per 10 millions. The first general observation is that in sea-coast, sub-montane and montane localities in India where endemic goitre is unknown the iodine-content of the soil may be very high, very low or moderate in amount. The absence of endemic goitre from these localities is related to conditions other than the iodine-content of their soils.

The soils from the neighbourhood of Coonoor, 6,000 ft above sea-level, in the Nilgiri Hills (Madras), are particularly rich in iodine (Table I), goitre as an endemic is unknown in this neighbourhood, that is to say, there is no evidence whatever of endemic goitre or of its sequelæ (cretinism, deaf-mutism and idiocy) in the adult population. But thyroid swelling is not uncommon in school-children. In a recent examination of 1,305 school-children of European and mixed parentage, in this locality, I found 'incipient goitre,' as defined by Hercus(20) and his colleagues,\* in 24.1 per cent of girls and in 11.3 per cent of boys (607 girls and 698 boys having been examined). Small but well-defined goitres, causing 'definite alteration in the normal contour of the neck, readily detected on inspection and palpation,'(20) were present in 3.6 per cent of girls and in 0.57 per cent of boys. The second general observation is that the richness of the soil in iodine does not preclude the presence in school-children, residing at an altitude of 6,000 feet above sea-level, of detectable thyroid swelling, amounting in a minority of cases to

\* 'Those in which a thickened isthmus could be clearly seen on deglutition, and distinctly felt as a thickened band lying across the trachea.'

*Results of analyses of samples of soil, salt and water-concentrates for iodine-content*

Sample Number	Nature of sample	Iodine in parts per ten millions of sample	REMARKS
I	Soil	400	
II	do	300	
III	do	400	Soil rich in iron
IV	do	30	
V	do	30	
VI	do	less than 10	
VII	do	10	
VIIIb and IXb	Bangalore Soil	" " 10	
X	Clay	60	
XII	Soil	" " 10	
XIII	do	10	
XIV to XVII	do	" " 10	
XVIII and XIX	do	10	
XX to XXVII	do	" " 10	
XXVIII	Salt	" " 10	
XXIX	do	" " 10	
XXX	do	" " 10	
XXXI	Water concentrate	trace	
XXXII	do	nil	
XXXIII to XXXV	do	Not estimated	
XXXVI to XLI	Soil	less than 10	

**B RESULTS OF IODINE ESTIMATIONS IN SOILS, SALTS AND WATER-CONCENTRATES**

The results arrived at by the three methods just described are given in the Tables I, II and III, wherein they are arranged in an order to facilitate discussion of their significance with respect to endemic goitre

that the highest figure is nearest the truth then the soil of this village is definitely richer in iodine than the soils of the other eight villages situated on the same alluvial fan, and this circumstance may be held by some to account for the freedom of this village from goitre. But the amount of iodine in the soil of Barmis is low, as low in fact as in the soils of other parts of the world, where 'goitre' prevails, as in New Zealand(20). Thus —

	Incidence of goitre per cent	Iodine in soil, parts per 10,000,000
North Otago	17	13
Southland and Otago	26·6	13
West Coast	52	12
Taumararua	35	14

It would seem, therefore, to be improbable that an iodine-content of from 5 to 15 parts per 10 millions in the soil of the Barmis village is in itself sufficient to account for the freedom of this village from goitre, more especially as Table I affords an example (V) of a place where goitre prevailed although the amount of iodine in its soil was considerably higher than that of Barmis.

(c) *In the third group* there are two soils (XXIV and XXV) from villages, situated in the heart of the endemic zone, but in which goitre is of very slight or slight prevalence, occurring only in females and in comparatively few of these. In regard to the first the iodine-content of the soil is as low as in other parts of the Gilgit Agency where goitre prevails. In regard to the second the iodine-content of the soil is approximately the same (4 to 8 parts per 10 millions) as in the village of Miragram (XXXVII) (5 to 9 parts per 10 millions), which is one of the most goitrous villages in Himalayan India(27). Both soils are poorer in iodine than that of Sanawar (V) where goitre prevailed. The comparative freedom of these two places from goitre is not, therefore, due to the richness of their soils in iodine.

(d) *The fourth group* includes the remaining sixteen samples of soil, the seventeenth sample (X)—being clay used in the Chin Hills, Burma(28), as a local application for the cure of goitre—will be considered separately. Fifteen of these soils are from selected villages in Chitral and Gilgit (Himalayas) in which goitre prevails with varying degrees of intensity and in which my original investigations of the disease were carried out (1902 to 1911). The two remaining samples (IV and V) are from Sanawar, Punjab (Himalayan foot-hills), where goitre had been endemic for many years(26) but which is now free from it, following, as already stated, the introduction of a new water-supply from the neighbouring station, Kasauli(11). All the soils from Chitral and Gilgit are very poor in iodine, their iodine-content ranging from less than 2 parts to 9 parts per 10 millions of soil. In general, then, it may be said that in these two localities of Himalayan India endemic goitre is commonly associated with a low iodine-content of the soil, but it is not always present in villages whose soils are

XXIV	Gakuch, Gilgit		less than 5	less than 2	" "	10	Third group — In endemic zone, only very occasional cases of goitre seen in females Fourth group — Endemic severe
XXV	Yasin, Gilgit		" 8	4	" "	10	
X	Clay spring, Chm Hills	45	38	28		60	A clay used as a local application for cure of goitre
IV	Sanavar garden	variable	8	4 5		30	Goitre formerly very prevalent
V	Sanavar hillside	25—50	35	19		30	Now disappeared
XVI	Basin, Gilgit	11 8	less than 5	2	less than 10		Villages of the Gilgit Fan, located on the same water-supply, goitre increases in incidence from the highest village (Basin) on the water-supply to the lowest (Kashrote)
XVII	Umpharis, Gilgit	20	" 5	less than 2	" "	10	
XIX	Domyal, Gilgit	18 8	" 5	3	" "	10	
XVIII	Majunphori, Gilgit	20	" 5	10	" "	10	
XX	Sonyar, Gilgit	24 5	" 5	3	" "	10	
XV	Kashrote, Gilgit	45 6	" 5	traces	" "	10	Congenital goitre and cretinism are very prevalent in the majority of these villages
XXI	Danyar, Gilgit	65	" 5	less than 2	" "	10	
XII	Nagar, Gilgit	10	" 5	4	" "	10	
XXVI	Banji, Gilgit	15	5	6	" "	10	
XXVII	Astor, Gilgit	45	" 5	" 2	" "	10	
XXXVI	Buni, Chitral	15	" 5	" 2	" "	10	
XXXVII	Miragram, Chitral	56 5	9	5	" "	10	
XXII	Awl, Chitral	47	" 5	" 2	" "	10	
XXIII	Sanoghar, Chitral	41 8	5	" 2	" "	10	

A Major Newcomb's results, B Mr Viswanath's results, C Dr Norris's results

All percentages shown in column 4 represent incidence of goitre in general population Only well-defined goitres included

for it Twenty-five years ago in Chitral the scarcity of salt in such villages was a complaint one often heard

Table II shows the iodine-content of two (XXVIII and XLI) substitutes for salt—*muniki mitti*—used by the people of Hunza and Nagar, respectively(10). The former contains 18 to 20 parts of iodine per 10 million parts of the material, the latter 5 to 6 parts. These substitutes for salt have been in use from time immemorial, and it would seem to be something more than mere coincidence that the people of Hunza who use the more iodine-rich material are free from goitre while the disease is becoming more prevalent in Nagar. But the matter is not quite so simple as this, for until 35 years ago Nagar was as free from goitre as Hunza now is [I have described its appearance in the former place elsewhere(10)] So that a *muniki mitti*, containing 5 to 6 parts of iodine per 10 million parts of the material, which may with some show of reason be credited with the prevention of goitre until 35 years ago, failed to prevent it under conditions of pollution of the main water-supply of this village

In so far, then, as iodine-containing salt and substitutes for salt are concerned there is reason to believe that they exercise a disfavoured influence on the development of this type of endemic goitre, but in certain circumstances this influence may not suffice wholly to prevent its occurrence

#### C THE IODINE-CONTENT OF WATER IN RELATION TO ENDEMIC GOITRE

The samples dealt with are five in number (Table III). They were selected with the object of settling certain specific points left in doubt by previous investigations —

- (a) Could the endemic occurrence of goitre in the Sanawar School, Punjab, be correlated with a low iodine-content of its water-supply? And was the disappearance of goitre from this school, following the introduction of a new water-supply from Kasauli, due to a greater iodine-content of the latter?
- (b) Was the freedom from goitre of the village of Barmis (Gilgit Fan) related to the iodine-content of the spring water used by the villagers? And
- (c) Was the incidence of goitre in the other eight villages of the Gilgit Fan (situated one above the other on the same water-supply) related to the poverty of this water in iodine?

##### (a) THE WATER-SUPPLIES OF SANAWAR

The history of goitre in this school has been fully dealt with in another place(26)(11), here it is enough to say that the disease was shown to be due to the consumption of a grossly polluted water, that the substitution of a bacteriologically pure water caused its complete disappearance, and that the new water contained less iodine than the old. It is learned from this experience that a water which contains as much iodine as 300 parts per 100 billion parts of the drinking-water (Table III) will not prevent goitre in the users of it when it is of a high degree of bacteriological impurity. Whether or not the bacteriological impurity must be of a specific nature to ensure this result is not definitely known,

TABLE III  
Showing the results of iodine determinations in Water-concentrates and the incidence of goitre

Sample Number	Source of sample	Endemicity of goitre per cent	Iodine in parts per 10 millions of drinking-water			REMARKS
			A	B	C	
XXXI	Sanawar, old supply	variable 25 to 50	0.03	0.0225	traces	This water formerly caused goitre in Sanawar
XXXII	Sanawar, new supply	nil	0.03	nil	nil	This water caused goitre to disappear in Sanawar
XXXIII	Barmis spring, Gilgit	nil	0.12			This water failed to prevent experimentally produced goitre in man
XXXIV	Main supply, Gilgit at source	118	7.06			These results are uncertain, they suggest, however, a loss of iodine by the water in its course through the villages of the Gilgit Fan
XXXV	Ditto at exit from last village	45.6	less than 0.021			

A Major Newcomb's results, B Mr Viswanath's results, C Dr Norris's results  
Percentages given in column 4 represent incidence of goitre in general population Only well-defined goitres included

course to the villagers became contaminated neither from these fields nor by human nor animal excreta

All nine villages have poverty of iodine in the soil as a common feature. In regard to the eight on the main water-channels there are no material differences (Table I) which would indicate that the soil of any one village was significantly poorer or richer in iodine than that of any other. Two observers found slightly more iodine in the soil of Majinpharri than in the soils of the other villages, the third found no more iodine in this soil than in the others. If we assume that the soil of this village did actually contain more iodine than the others then its greater richness in iodine did not lessen the incidence of goitre in the general population to any extent. The significance of the appreciably greater iodine-content of the soil of the ninth village (Barmis) has already been discussed. It would seem, therefore, that the increasing incidence of goitre in the eight villages located on the main water-supply of the Gilgit Fan was not related to the iodine-content of their soils.

Chemical analysis of the soils of these villages has failed to reveal any other chemical substance, which would have accounted for the increasing incidence of Goitre (Table V). The one possible factor which might have been suspected of such an influence was sulphate—a gradual increase occurs in the sulphate-content of the soil from the highest village to the lowest. But this appears to be of no significance, since the soil of Barmis, where goitre did not occur, was as rich in sulphate as that of Kashrote where the disease had its highest incidence.

TABLE V

*Showing the chemical composition of the soils of the villages on the Gilgit Fan*

	Barmis	Basin	Kashrote	Umpharis	Damyal	Majinpharri	Sonyar
Water (at 110°C)	0.751	0.945	0.76	1.00	0.871	1.4	3.50
Loss on ignition	2.38	1.54	2.04	3.15	3.025	4.96	0.935
Organic matter	1.63	0.595	1.28	2.15	2.15	3.51	2.57
Total insoluble matter	82.40	82.57	80.65	85.63	81.61	78.78	83.30
Nitrogen (total)	0.114	0.148	0.133	0.145	0.109	1.160	0.1194
Alumina (Al <sub>2</sub> O <sub>3</sub> )	9.82	5.63	6.33	2.71	7.07	7.43	6.19
Ferric oxide (Fe <sub>2</sub> O <sub>3</sub> )	3.37	3.80	4.78	4.69	4.44	3.78	4.83
Lime (CaO)	2.55	1.23	2.656	1.33	2.59	1.82	1.62
Magnesia (MgO)	0.61	2.09	0.542	1.95	0.73	2.22	0.91
Potash (total) (K <sub>2</sub> O)	0.927	0.679	0.716	0.48	0.972	0.642	0.626
Phosphoric acid (P <sub>2</sub> O <sub>5</sub> )	0.018	0.039	0.051	0.168	0.032	0.28	0.098
Sulphuric acid (SO <sub>3</sub> )	0.19	0.048	0.084	0.08	0.097	0.131	0.190
Carbon dioxide (CO <sub>2</sub> )	0.024	0.172	2.22	0.10	0.124	1.27	1.06

noticeable goitre, although goitre, as an endemic may be conspicuous by its absence in the general population. The majority of such swellings (i.e., the so-called 'incipient goitres') are in the main physiological and have, in this locality, no title whatever to be classed as 'goitre'—a term which signifies thyroid disease, the minority are true goitres but they bear no relation to the iodine-content of the soil.

Three garden soils (XXXVIII, XXXIX and XL) from the neighbourhood of Madras city contained less than 5 parts of iodine per 10 millions parts of soil. Goitre is unknown as an endemic in this neighbourhood, yet the poverty of the soil in iodine is as great as in some of the most goitrous regions of Himalayan India where the disease and its sequelæ prevail with great intensity. The neighbourhood under reference is, however, near the sea-coast, but proximity to the sea-coast does not preclude the occurrence of goitre though it may not be of the same type as that prevailing in mountainous regions, while the soils of a number of sea-coast localities in other parts of the world, where 'goitre' is endemic, have been found to contain considerably more iodine than these three soils of Madras. It follows, therefore, that the poverty of iodine in the soil of this neighbourhood causes neither childhood goitre nor endemic goitre of either type.

(b) In the second group there are four soils (VI, VII, XIII and XIV) all very important. VI and VII are from Kasauli, Punjab(26), in which endemic goitre\* in school-children is 22 per cent—a figure representing the usual incidence of childhood goitre in non-endemic localities in India, and one which is independent of endemic influences. The iodine-content of these two soils is approximately the same as that of two soils from the neighbouring hill of Sanawar (IV and V) where, until a few years ago, goitre was endemic(26), it has now disappeared as a result of the substitution of the bacteriologically pure water-supply of Kasauli for the bacteriologically impure water-supply of Sanawar(11). Kasauli and Sanawar are about 3 miles apart. It follows, therefore, that in the foot hills of Himalayan India two places adjacent to one another may have approximately the same iodine-content in their soils yet goitre be endemic in the one and not in the other.

The other two soils in this group (XIII and XIV) are both from localities situated in the heart of the endemic zone in the Gilgit Agency. In regard to the first (XIII)—that obtained from Hunza—the freedom of Hunza from goitre is certainly not related to the richness of its soil in iodine, since it contains less than 5 parts per 10 millions or about the same amount as the soils of other places in the Gilgit Agency where endemic goitre prevails with great intensity. The second soil (XIV) is from the village of Barmis on the Gilgit Fan(27). Goitre is not endemic in this village, although it prevails in the other eight villages on this fan (vide *infra*), the only cases of the disease met with being imported from outside villages. The iodine-content of the soil of this village is variously estimated at from 5 to 15 parts of iodine per 10-million parts of soil (Table I). Assuming

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\* I have never in my investigations included the so-called 'incipient goitres' of childhood—  
R. McC.



above the other on the same water-supply, the effluent from one tank flowing into the tank next below it in the series Gaylord recorded an incidence of goitre in the fish as follows —

Water above the tanks	Fish goitre-free
„ in 1st tank	3 per cent goitrous
„ „ 2nd „	8 „ „
„ „ 3rd „	45 „ „
„ „ 4th „	84 „ „

Marine provides similar figures, both observers finding that the water above the tanks was not goitre-producing, thus 'Fish which lived in the raceway above all houses and which had never been confined in the tanks maintain normal thyroids throughout their lives' (Marine) Now the type of goitre occurring in these fish was the same as that occurring in the villagers of Gilgit a condition of sustained hypertrophy (or 'hyperplasia' as it is sometimes called) of the thyroid gland And the causes which give rise to it, or rather the summation of causes, were of like order in both man and fish That these causes were related to the water-supply—the medium in which the fish lived—there can be no reasonable doubt It is equally clear that whatever the causes of the goitre may have been it was not due to the absence of iodine from the water-supply either of the trout or of the Gilgit villagers since fish living in the water above the tanks were goitre-free, and a water (XXXII) which contained little or no iodine has been shown to have been capable of causing the complete disappearance of the disease

We have seen that the goitre both in man and in trout was a compensatory hypertrophy This in itself indicates that the gland must have been receiving enough iodine to enable its complete cycle of functional activity to take place, for as Williamson and Pearse(17) have pointed out it is not possible to conceive of a productive hypertrophy arising from the absence of an element (iodine) which is an essential constituent of the secretion to be produced by that hypertrophy Nevertheless, it is conceivable that this type of goitre may be related in its origin to a deficiency of iodine relative to the needs of the body for the gland's iodine-containing secretion It is not that the thyroid gland has not enough iodine for all normal needs but that it requires more for abnormal needs It is these abnormal needs which provide the stimulus causing the gland's hypertrophy and which constitute the essential cause of this type of goitre These needs are engendered in trout by their conditions of life in unhygienic tanks or ponds, and in man by his conditions of life in unhygienic villages In the trout the severity of the disease 'is quantitatively related to the general hygienic conditions prevailing, and to the food, water-supply, and degree of crowding' (Marine) In the Gilgit villagers it is the summation of factors of a like order which determines the severity of the endemic In such circumstances the removal of 'the last straw' enables the overburdened thyroid to regain a more normal poise, but the straw removed need not always be the same straw It may be one represented by the bacteriological impurity of the water or one represented by a particular ingredient of the food or by some other factor This truth is exemplified by the disappearance of goitre from the Royal Military School, Sanawar, consequent on

as poor in iodine as those of other villages in which the endemic prevails, nor does the incidence of goitre in the latter villages appear to be in inverse ratio to the amount of iodine in their soils

The sixteenth sample, from Sanawar (V), provides an example of a soil having a considerably higher iodine-content than any of the others in this group Yet so potent were endemic influences in Sanawar a few years ago that 8 years residence there resulted in 80 per cent of the school-children becoming goitrous(26)

From the foregoing facts it would appear that while the type of goitre with which we are dealing is prone to be associated with a low iodine-content of the soil, this does not in itself determine, so far as Himalayan India is concerned the presence of the disease nor control its incidence This control is exercised by other influences which are not related to the iodine-content of the soil

## B THE IODINE-CONTENT OF SALT IN RELATION TO ENDEMIC GOITRE

Table II gives the results of iodine-estimations of five samples of salt or of substitutes for salt used in various places where goitre is endemic The possible influence of these in *preventing* goitre has now to be considered Sample XI is of peculiar interest This 'salt' is made by the evaporation of the water of the Nat-ye-Chaung spring, in the Chin Hills, Burma I have referred to it on another occasion(28) but at that time I was not aware of the large amount of iodine it contains (70 to 75 parts per 10 million parts of the salt) This salt has a high local repute for the cure of goitre With it the people use the clay from the banks of the stream as a local application to their goitres The clay (Table I, sample X) contains from 28 to 38 parts of iodine per 10 millions Dr Rodriguez, Civil Surgeon, Chin Hills, noted (1913) the very interesting fact that though goitre was common in most villages in the Chin Hills there was 'an area in which were some half-dozen villages, all within a radius of five miles, absolutely free from goitre Further inquiry led (him) to a spring (the Nat-ye-Chaung) from which these villages made their "salt" by evaporation of the spring water Of course the quantity was small and they had to supplement their requirements with the salt imported from Burma'(28) It would seem reasonable to suppose that the use of the salt, made from the water of this spring, contributed to the freedom of these villages from goitre, although there may also have been other causes of their immunity of no less importance than the iodine-containing salt

Practically the only imported food material used by the general population of Gilgit is 'rock salt' which, as seen from Table II, contains only traces of iodine or less than 2 parts per 10 millions 'Traces of iodine' in salt have sometimes been credited with the prevention of goitre as, for instance, in the Canton of Vaud, but the traces in the rock-salt in common use in Gilgit did not suffice to do so Nevertheless, this salt may have had some effect in causing the lesser incidence of the disease in the richer than in the poorer classes, since the poor commonly find it difficult to secure a sufficiency of this essential ingredient of the food, and in the more remote parts of Himalayan India they have often to resort to substitutes

is of exceptional purity and well protected, and in the latter the amount of iodine in the soil is high. In Kasauli I had no difficulty in producing goitre in experimental animals by confining them in dirty wooden cages, but in Coonoor I have only succeeded in doing so by adding to the food of the animals some ingredient, such as an excess of butter, which imposes an additional burden on the gland. In Kasauli it required the most scrupulous cleanliness to prevent the disease, in Coonoor much less care was needed, although the effects on the gland of unhygienic conditions of life were evidenced by a general increase in its size amounting in one experiment to over 50 per cent. It is concluded, therefore, that the adeno-parenchymatous type of endemic goitre is less likely to arise in localities where the soil and water-supply are rich in iodine than in those where they are poor in iodine. In the latter I have prevented the disease arising under experimental conditions in animals by the additional provision of iodine in their food (33) the provision of more iodine easing in some way the strain put upon the thyroid gland by the peculiar conditions of life under which this type of goitre arises.

At the present time there are those who find in iodine-deficiency an all-sufficient explanation of 'goitre,' forgetful, perhaps of the variety of its types, others who will have nothing to do with this view forgetting that the functional perfection of the thyroid gland—its initiation and its maintenance—is dependent, amongst other things, on the adequate provision of iodine for every eventuality (33). Iodine is in a sense the lubricant of the thyroid engine, though it is more than this since it is an essential component of thyroxine, and, it would seem that the more work the thyroid engine has to do the more lubricant, within limits, it needs. But overwork of the thyroid engine—and it is with this aspect of its activities that we are here concerned—is not merely a matter of lubrication, but of its capacity as a machine, its physiological efficiency, its state of nutrition, the state of the gastro-intestinal tract (governing its nutrition and the supply to it of the ingredients—not iodine alone—from which it elaborates its products), its nervous control, the nature of the work it is called upon to perform and the impediments, intrinsic and extrinsic to the gland, that are placed in the way of its performance. In the study of the 'goitres' all these things have to be taken into consideration no less in regard to the endemic goitres than in regard to those that are sporadic. For there can be no doubt that when we argue about the 'causation of goitre' we are not always arguing about the same disease, nor that the constellation of causes which may give rise to one type of thyroid change is necessarily always the same in every part of the world nor the progress of this change always the same in every locality.

### SUMMARY

(1) The investigations dealt with in this paper relate only to the classical or adeno-parenchymatous type of endemic goitre.

(2) In sea-coast, sub-montane and montane localities (ex Himalayan India) where endemic goitre is unknown, the iodine-content of the soil may be low, moderate in amount, or high. The freedom of these localities from goitre is related to conditions other than the iodine-content of their soils.

but animal experimentation has thrown strong suspicion on intestinal bacteria, especially intestinal anaerobes (29)

Recently (1926) Keith (36) has reported that the incidence of goitre in the Pemberton valley, B. C. is 80 per cent although the drinking-water contains 103 parts of iodine per million, whereas there is only 8 per cent of goitre in Vancouver where the drinking-water contains no detectable iodine

(b) (c) THE VILLAGES OF THE GILGIT FAN

My original account of the epidemiological survey of endemic goitre in these villages was published in 1906 (27). It has since been widely quoted in the literature, sometimes accurately more often inaccurately. It is necessary briefly to refer to it here. There are nine villages on this fan, eight being situated one above the other on the main water-supply, the ninth is provided with its own supply. Goitre is prevalent in the first eight villages, it does not prevail with equal intensity in all, but shows an increase in incidence from that highest to that lowest on the water-supply.

TABLE IV

*Showing the incidence of goitre in the villages of the Gilgit Fan*

Sample Number	Village	Population	Number of houses	Number of houses having goitrous inhabitants	Per cent of houses having goitrous inhabitants	Per cent of goitrous persons in houses having goitrous inhabitants	Per cent of total population goitrous
XVI	(1) Basn	93	15	9	60	21.2	11.8
XVII	(2) Umpharis	385	66	42	63.6	28.3	20
XIX	(3) Damyal	181	30	20	66.6	30.3	18.8
XVIII	(4) Majinpharri	718	108	68	63.2	24.2	20
—	(5, 6) Kyk	229	33	23	71.5	30	26.9
XX	(7) Sonyar	458	63	52	82.5	30	24.5
XV	(8) Kashrote	128	24	21	87	36	45.6

The increasing incidence of the disease was confined to persons over the age of 15 years, so much so that had children under this age been removed from the calculation it would have appeared more regular and striking. It was correlated by me with the increasing impurity of the water in the open channels which acted not only as conduits of drinking-water but as irrigation-channels and open drains.

The ninth village (Barmis) situated at the 'handle' of the fan, was goitre-free. It received its water-supply from a spring of exceptional purity, so located with respect to the village and to its irrigated fields that the water in its

and water concentrates for our iodine-determinations Without their help the work could not have been done

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The water-supply of the Barmis village contained 1,200 parts of iodine per 100 billion parts of drinking-water (0.12 parts per 10 millions) (Table III), a circumstance which may have been concerned in the freedom of this village from goitre, since the amount of iodine in the water is of the same order of magnitude as that in waters derived from iodine-rich localities in the United States of America (McClendon and Hathaway) (30). While admitting that the higher iodine-content of both the water and the soil of this village is significant and may have been in some part responsible for its freedom from goitre, yet I have evidence that the bacteriological purity of its water-supply was probably a factor of no less importance. I have recounted in another place my experiments on young men designed to produce goitre by means of the ingestion of the suspended matter removed by filtration from the main water-supply of the Gilgit villages at its exit from the last one, Kashrote (1) (10). These experiments were conducted under conditions of the most rigid military discipline, the men (with their controls) being encamped in the neighbourhood of the village of Barmis and supplied with water from the Barmis spring for drinking, culinary and other purposes. The use of this water did not prevent 10 out of 36 of these young men (including myself) developing well-marked enlargement of the thyroid gland nor 5 others from showing swellings of the organ of a transitory nature, while 31 who consumed the same suspended matter in the same amounts after it had been boiled showed no swelling of the gland. It is obvious that this water, although it contained 1,200 parts of iodine per 100 billion parts of water, failed to prevent thyroid hypertrophy in 41 per cent of young men, aged between 18 and 22 years, when they consumed daily large quantities of the unboiled suspended matter from the goitre-producing water of Kashrote. I consider, therefore, that the freedom of the Barmis spring-water from pollution was an important factor determining the freedom of this village from goitre. This experience emphasizes a point of fundamental importance in regard to the type of endemic goitre with which we are dealing: the disease in certain circumstances can arise despite the presence in the drinking-water of considerable amounts of iodine.

Major Newcomb is doubtful whether the water of the other eight villages, at its source, contains any iodine, 'but if any then not more than 0.2 parts per million parts of the water concentrate' or 600 parts per 100 billion parts of the drinking-water (Table I). It was unfortunate that the difficulties of procuring samples of water-concentrates from these inaccessible places were so great and that such large amounts of water have to be concentrated in order to detect small amounts of iodine, for we are left still in doubt as to the actual iodine-content of this water at its source, and in doubt also as to whether it loses iodine on its way down from the highest to the lowest village on the Fan, although his results suggest that it may do so. It seems certain, however, that this water at its source contained little iodine, but this does not in itself render a water goitre-producing since sample XXXII, which also contained little or no iodine, caused the disappearance of goitre from Sanawar.

Following the publication of my original account of goitre in these villages, Marine and Lenhart (1910) (31) and Gaylord (1912) (4) recorded precisely similar observations in artificially-bred trout living in tanks or ponds situated one

In working with diets deficient in vitamins I have hitherto noted a general tendency to diminution in size of the thyroid gland as a result of the dietary deficiencies, (4) (5) (6) as for instance, in pigeons fed on polished rice, or on autoclaved rice with or without the addition of butter or of onions or of both, and in monkeys fed exclusively on rice diets or on mixed diets the vitamin-B-value of which had been reduced by autoclaving. But in looking through my records of over 2,000 animals fed on deficient diets of various kinds I find occasional exceptions to this rule. One diet composed entirely of rice obtained from the neighbourhood of the Chin Hills in Burma (where goitre is endemic) and another, composed entirely of American white-flour, were associated with thyroid enlargements sufficiently often to make this association significant. Another exception was provided by guinea-pigs fed on crushed oats and autoclaved milk (4). This diet caused marked thyroid enlargement of a congestive kind, its chief faults were deficiency of vitamin-C, of vegetable salts and of roughage.

But although there is a general tendency to diminution in size of the thyroid gland in animals fed on the deficient diets referred to, the small-sized organ is commonly of the 'actively secreting type' (5) (7) showing heightened epithelium, depletion of the gland's store of colloid material and vesiculation of the epithelial columns by secretion proper—a condition of affairs in sharp contrast to that brought about by starvation, wherein the epithelium of the acolloid thyroid shows no signs of increased secretory activity (8). These changes appear to represent a response on the part of the gland to some metabolic need or stimulus, but they are by no means so constant a consequence of vitamin-deficiency, nor do they result in thyroid enlargement (goitre) so often, as to justify the conclusion that the vitamin-deficiency is the sole factor concerned in the production of the goitres which occasionally result in animals fed on foods having this defect.

In view of the comparatively wide prevalence of 'simple goitre' amongst white-flour-eating peoples and of the prominence which has of late years been given to iodine-deficiency as its sole cause and to iodine-prophylaxis as the only means of its prevention, it was thought desirable to ascertain whether diets into which white-flour entered largely, and from which green vegetables and fruit were excluded, could give rise to, or favour the development of, 'goitre' in animals receiving iodine in adequate amounts and living under conditions of the strictest hygiene.

#### DETAILS OF THE EXPERIMENT

Sixteen groups of young rats from non-goitrous stock, six animals in each group, were selected for this work, males and females being as far as possible equally distributed in each group and the age of the animals being approximately the same (30 to 60 days). Their initial weights ranged from 30 to 67 grammes. Each animal was confined in a separate cage so constructed as to render hygienic conditions of life as perfect as possible. The cages were provided with netted-wire bottoms of fine mesh through which the excreta fell into zinc trays below. During feeding-time clean trays were passed into slots above the netted-wire bottoms of the cages so as to prevent loss of food, at the same time attendants

the substitution of a pure for an impure water-supply although the new supply contained less iodine than the old(11) Clearly, in this case, the essential or exciting cause of the disease was not want of iodine in the drinking-water but the constant ingestion with the polluted water of bacterial agents or their products Similarly, so small a change in the constitution of the food as the substitution of skimmed milk for whole milk, although the latter contains more iodine than the former, has sufficed to prevent the congenital manifestations of this type of endemic goitre in pigs(32), the reason being the reduction in the intake of fats and the relief afforded to the thyroid gland which is thereby brought about

Goitre may be caused to disappear by still other means Marine and Lenhart(7) brought about its disappearance in artificially reared trout by the addition of small amounts of iodine to the water in which the fish lived This result has been erroneously interpreted to mean that the cause of the disease was the initial poverty of the water in iodine(34), whereas it has been clearly shown that any poverty in iodine which may have existed did not suffice to cause the disease in fish living in the water outside the tanks As well might the goitre be attributed to a deficiency of perchloride of mercury in the water since Gaylord has found the addition of minute traces of this substance to be equally effective in preventing and curing the disease(4) The prevention of goitre by these means does not indicate that poverty either of iodine or of mercury in the water is the essential cause of the disease but it does appear to indicate that the *additional* provision of iodine enables the thyroid gland to satisfy more easily the demands made upon it when fish are living in unhygienic surroundings, and, that the addition of perchloride of mercury to the water lessens these demands by limiting the growth of bacteria and the production of their toxins both in the tanks and in the intestinal tract of the fish It is remarkable how little attention has been paid in the literature to Gaylord's important observation, the tendency ever being to extol the influence of iodine as a preventative and of its want as a cause of the disease, thereby minimizing the importance of other factors in its causation, and limiting the whole question of its ætiology to a too narrow field

It has been shown that the essential cause of goitre in the villagers of Gilgit was not the poverty of the soil in iodine It is, I think, equally clear that its essential cause was not the poverty of the drinking-water in iodine, but the unhygienic conditions of life in the villages Among these conditions bacteriological impurity of the drinking-water was the chief, since the massive ingestion of the unboiled suspended matter in this water caused the disease under experimental conditions in man, despite the use of a drinking-water which contained 1,200 parts of iodine per 100 billion parts of water But I now realize that bacteriological impurity of the water-supply may not *per se* suffice to cause this type of endemic goitre unless the impurity be of a specific kind, which is yet unproven, or unless the amount of iodine ingested with the food and water be relatively small, or the amount of harmful bacteria and their products ingested be relatively large This conclusion has been impressed upon me by noting the differences in my experimental results in two localities—Kasauli and Coonoor—the one poor in iodine the other rich in it (Table I) In neither place is endemic goitre prevalent, for the reason, apparently, that in the former the water-supply



*Goitre Unrelated in its Origin to Iodine*

TABLE I  
Feeding details regarding the 96 animals used in these experiments

Group Number of Diet	Laboratory Number of Animal	Sex	Initial body-weight in grammes	Maximum weight attained in grammes	Final body-weight in grammes	Days under experiment	Amount of potassium iodide consumed daily in mgrms	Cause of death.	Goitre
I	535	M	41	55	52	104	nil	Pneumonia	No
	536	F	50	67	53	120	nil	Asthenia	No
	537	M	50	49	41	68	nil	Asthenia	No
	538	F	40	45	45	23	nil	Asthenia	No
	539	M	50	61	61	28	nil	Asthenia	No
	540	F	40	46	40	130	nil	Asthenia	Yes
II	529	M	45	68	55	127	nil	Asthenia	Yes
	530	M	40	67	46	128	nil	Pneumonia	No
	531	F	43	70	53	90	nil	Asthenia	Yes
	532	M	46	69	51	130	nil	Pneumonia	No
	533	F	47	65	47	90	nil	Asthenia	No
	534	F	40	44	33	75	nil	Asthenia	Yes

(3) The richness of a soil in iodine does not preclude the presence of thyroid swellings (mostly so-called 'incipient goitres') in 27·7 per cent of girls and in 11·8 per cent of boys living at an altitude of 6,000 ft above sea-level and in a place where endemic goitre is conspicuous by its absence in the general population

(4) In Himalayan regions where the adeno-parenchymatous type of goitre is endemic the iodine-content of the soil is in general very low, but the disease is not always endemic in villages whose soils are as poor in iodine as those of other villages in which the endemic prevails.

(5) The investigation has provided no evidence that in Himalayan India the incidence of endemic goitre is in inverse ratio to the iodine-content of the soil. In the heart of the endemic zone, as well as in the Himalayan foot-hills, two places adjacent to one another may have approximately the same amount of iodine in their soils yet goitre be endemic in the one and not in the other, or, the soils of two villages situated on the same water-supply may be equally poor in iodine yet goitre be four times as prevalent in the one as in the other.

(6) The water-supply of a given place may contain an appreciable amount of iodine and yet goitre be endemic therein, the water-supply of another may contain less iodine and yet goitre may not be endemic therein.

(7) Drinking-water containing 300 parts of iodine per 100 billion parts of water has not prevented endemic goitre in the presence of a high degree of bacteriological impurity of the water, nor has the consumption of a drinking-water containing 1,200 parts of iodine per 100 billion parts of the water prevented the development, under experimental conditions, of this disease in young men who consumed large amounts of the suspended matter removed by filtration from a grossly-polluted, goitre-producing water.

(8) The substitution of a bacteriologically pure for a bacteriologically impure water has caused the rapid disappearance of the endemic although the new water-supply contained less iodine than the old. The bacteriological impurity of the water-supply was the essential cause of the disease.

(9) Although the iodine-content of the soil and water is not in itself the essential cause of this type of endemic goitre in Himalayan India, yet the disease is less likely to arise under experimental conditions in iodine-rich localities in Southern India than in iodine-poor localities in Himalayan India.

(10) Iodine-containing salt or substitutes for salt appear to have an influence in preventing this type of endemic goitre. But in certain circumstances their use does not prevent it.

(11) The essential cause of this type of endemic goitre in Himalayan India is the unhygienic conditions of life of the people. Among these conditions bacteriological impurity of their drinking-water and of their surroundings is the chief. A deficiency of iodine relative to these conditions of life is favourable to the development of the disease.

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TABLE I—*contd*

Group Number of Diet	Laboratory Number of Animal	Sex	Initial body-weight in grammes	Maximum weight attained in grammes	Final body-weight in grammes	Days under experiment	Amount of potassium iodide consumed daily in mgrms	Cause of death	Goitre
VI	505	M	50	58	50	71	13 to 26	Asthenia	No
	506	F	40	139	139	165	15 to 26	Killed	Yes
	507	F	40	115	115	165	15 to 26	Killed	Yes
	508	F	48	93	93	165	12 to 26	Killed	No
	509	M	50	86	48	70	10 to 26	Pneumonia	No
	510	F	43	107	107	165	11 to 26	Killed	No
VII	499	F	40	61	41	75	13 to 26	Asthenia	Yes
	500	F	50	94	60	87	04 to 26	Asthenia	No
	501	M	43	139	139	165	15 to 26	Killed	No
	502	M	48	87	50	74	10 to 26	Asthenia	No
	503	F	41	66	40	73	04 to 26	Asthenia	No
	504	M	49	61	43	70	13 to 26	Asthenia	No
VIII	427	F	30	75	75	100	06 to 195	Killed	No
	428	M	67	107	107	100	08 to 195	Killed	No
	429	F	50	99	99	100	07 to 195	Killed	No
	430	F	38	92	89	100	11 to 195	Killed	No
	431	M	55	110	93	86	06 to 195	Paralysis	No
	432	M	32	90	88	100	04 to 195	Killed	No

# THE EXPERIMENTAL PRODUCTION OF A NEW TYPE OF GOITRE UNRELATED IN ITS ORIGIN TO IODINE

BY

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## INTRODUCTION

THE goitres hitherto produced by me under experimental conditions in animals(1) (pigeons, rats and goats) have been of three types the hypertrophic the hyperplastic and the colloid. The first may arise in animals living under unhygienic conditions of life in iodine-poor localities, it arises also in animals receiving massive doses of faecal bacteria in admixture with their food, congenital goitre and cretinism being thereby produced in their offspring. The second may arise in animals receiving an excess of fats or of fatty acids in an otherwise well-balanced diet (2). The third has not been produced in typical form as seen in man, but a condition simulating it in pathological features has resulted in animals from the excessive ingestion of lime in an otherwise well-balanced food (3). With the possible exception of those caused by the administration of cultures of faecal bacteria the goitres produced in these ways are preventable by increasing the intake of iodine proportionately to the unhygienic conditions of life of the animals or to the excess of fats or of lime in their food.

The present paper deals with the experimental production of a form of goitre which conforms to none of these types, which has distinctive ætiological and pathological features, and is unrelated in its origin to the iodine ingested. Its histological features are those of an intense secretory hypertrophy approaching that seen in Primary Graves's Disease. It has been produced in young rats by means of diets containing 60 per cent or more of American white flour, 20 per cent or less of protein, fats and salts (including iodine) in adequate amounts but no green vegetable foods nor fruit, that is to say by diets of which a principal fault is an insufficiency of vitamins of the A, B and C classes.

TABLE I—*concl'd*

Group Number of Diet	Laboratory Number of Animal	Sex	Initial body weight in grammes	Maximum weight attained in grammes	Final body weight in grammes	Days under experiment	Amount of potassium iodide consumed daily in mgrms	Cause of death	Goitre
XII	221	F	65	86	67	106	0.6 to 1.95	Killed	No
	222	F	48	70	46	83	0.8 to 1.95	Paralysis	No
	223	M	55	92	55	79	1.1 to 1.95	Paralysis	No
	224	F	45	78	76	106	0.7 to 1.95	Killed	No
	225	F	40	76	76	106	0.6 to 1.95	Killed	No
	226	M	37	98	95	106	1.1 to 1.95	Killed	No
XIII	403	F	35	46	34	46	0.7 to 1.95	Asthenia	Yes
	404	F	52	69	69	97	0.7 to 1.95	Asthenia	No
	405	F	45	61	42	56	0.8 to 1.95	Asthenia	Yes
	406	F	48	72	51	62	0.8 to 1.95	Asthenia	No
	407	M	47	54	35	41	0.7 to 1.95	Asthenia	No
	408	F	45	51	36	49	0.5 to 1.95	Asthenia	Yes
XIV	385	F	60	74	53	37	1.1 to 1.95	Paralysis	Yes
	386	M	50	69	53	48	0.8 to 1.95	Paralysis	Yes
	387	F	45	54	48	40	1.1 to 1.95	Paralysis	No
	388	F	39	49	40	47	1.3 to 1.95	Pneumonia	Yes
	389	F	45	53	44	40	1.2 to 1.95	Beri-Beri and Pneumonia	Yes
	390	F	33	53	34	41	0.8 to 1.95	Paralysis	No

went from cage to cage removing any excreta passed. The cages were washed with antiseptic lotion daily and were sterilized in boiling water at regular intervals in a specially constructed apparatus. (These precautions are absolutely essential in all experimental work on goitre in order that the factor of dirt may be excluded, this factor being of itself prone to cause thyroid changes, sometimes amounting to actual goitre, especially if the iodine-intake of the animals be low(1)(7)(10) ) At night the animals were bedded in sterilized cotton waste. Each was given a known quantity of its particular diet daily, the balance not eaten being weighed and the daily food consumption by each animal recorded (Table I). Distilled water was provided for drinking and washing purposes. Each animal was weighed weekly and the weights charted. Clinical notes of any symptoms shown by them were kept, and a complete post-mortem examination of each was made at the end of the experiment so as to ascertain the cause of death. In animals dying before the end of the experiment post-mortem examination was made as soon as possible after death in order to limit the autolytic changes to which the thyroid gland is so liable. To estimate the extent of any such changes a number of healthy well-fed young rats, of the same age as the experimental animals, were killed, their thyroid glands removed at 2, 4, 6, 8, 12, 24 and 36 hours after death, fixed, embedded, sectioned and stained by the same processes as those adopted in the study of the goitrous organs. In none of the experimental animals was post-mortem examination delayed for longer than 12 hours, in very few for so long. The animals surviving to the end of the experiment were killed and their thyroids removed and fixed immediately. The diagnosis of 'goitre' was made on macroscopical characters and subsequently confirmed microscopically. A number of non-goitrous thyroids were also examined for purposes of comparison with the goitrous, and in order to detect the earliest signs of the gland's departure from normal. Details regarding each animal are given in Table I.

#### THE EXPERIMENTAL DIETS

The materials used in the preparation of the experimental diets were American white-flour, purified starch, olive oil, 'meat-residue' and 'salt-mixture'. The white-flour was obtained from the local market, the starch from a firm of manufacturing chemists, the olive oil was Crosse and Blackwell's 'Lucca oil', the cod-liver oil was Allen and Hanbury's, and the yeast was dried brewer's. The 'salt-mixture' was made up as follows—calcium lactate, 39 grms, calcium phosphate, 16.2 grms, potassium phosphate, 28.62 grms, iron citrate, 3.54 grms, sodium chloride, 5.19 grms, magnesium sulphate, 7.98 grms, sodium phosphate, 10.41 grms, and potassium iodide, 0.5 grms (McCollum and Davies)(11). The 'meat-residue' was prepared by a method described elsewhere(12). The percentage composition of the diets is given in Table II. Table I shows the daily food consumption per rat in each group and the daily amount of potassium iodide ingested. These Tables also show the number of cases of goitre resulting from the different diets, while the effect of the diets on growth is shown in Table I.

TABLE II

*Showing the percentage composition of the experimental diets and the incidence of goitre*

Class of Diet	Group Number of Diet	Number of animals in group	American white-flour	Olive oil.	Salt-mixture	Meat residue	Starch	Cod-liver oil	Dried yeast	Number of animals goitrous	
A	I	6	100							1	Control Diet
	II	6	92	8						3	
	III	6	95		5					2	
	IV	6	87	8	5					3	
	V	6	82	8	5	5				3	
	VI	6	77	8	5	10				2	
	VII	6	72	8	5	15				1	
B	VIII	6	60	10	5	20				0	Control Diet
	IX	6	50	10	5	20	10			0	
	X	6	40	10	5	20	20			0	
C	XI	6	30	10	5	20	30			1	
	XII	6	20	10	5	20	40			0	
	XIII	6	10	10	5	20	50			3	
	XIV	6		10	5	20	60			4	
	XV	6		10	5	20	60			2	
	XVI	6		8	5	20	60	2	5	0	

#### INFLUENCE OF THE EXPERIMENTAL DIETS IN CAUSING GOITRE

There were 25 cases of thyroid enlargement among the 96 animals a case incidence of 26 per cent

The various diets range themselves into three classes according to their influence in causing goitre —

- (a) Those on which the white-flour-content is greater than 60 per cent and the protein-content less than 20 per cent, fats and salts being adequate (II to VII)

III	523	M	50	56	42	55	06 to 26	Scurvy	No
	524	M	41	68	56	54	04 to 26	Asthenia	Yes
	525	F	40	45	42	36	08 to 26	Pneumonia	No
	526	M	50	75	57	74	08 to 26	Asthenia	Yes
	527	F	40	60	40	66	07 to 26	Edema	No
	528	F	50	66	52	37	12 to 26	Pneumonia	No
IV	517	M	42	51	37	87	04 to 26	Asthenia	Yes
	518	F	47	67	52	75	08 to 26	Asthenia	Yes
	519	F	40	55	49	165	08 to 26	Killed	No
	520	F	50	56	39	73	10 to 26	Beri-beri	No
	521	M	42	51	39	66	05 to 26	Asthenia	Yes
	522	M	50	79	69	131	03 to 26	Asthenia	No
V	511	M	48	72	52	70	07 to 26	Pneumonia	No
	512	M	40	92	70	151	13 to 26	Pneumonia	No
	513	F	41	74	47	70	13 to 26	Asthenia	No
	514	M	43	89	59	75	04 to 26	Asthenia	Yes
	515	F	49	105	72	94	08 to 26	Asthenia	Yes
	516	F	50	80	63	143	11 to 26	Asthenia	Yes



therefore, reached that the pathogenesis of this type of goitre was independent of the amount of iodine ingested with the food

#### SEX-INCIDENCE OF THE GOITRE

Of the 96 animals used in this experiment 42 were males and 54 females. There were 9 cases of goitre in males, 16 in females, a case incidence of 21.4 per cent in males and 29.6 per cent in females. Taking into account only the goitre-producing diets (V, VI, VII and XI) which ensured fair growth in the young rats consuming them, there were 24 animals to be considered. Of these 13 were males and 11 were females. Goitre occurred in one male only, while 6 females were affected by it—a case incidence of 7.7 per cent in males and 54.5 per cent in females. Although the figures are small yet this difference in sex-incidence appears to be sufficiently marked to justify the conclusion that females are more likely to suffer from this type of goitre than males—a significant conclusion in view of the probable relationship of this type of thyroid change to Graves's Disease and Myxœdema (*vide infra*), and of the higher incidence of these conditions in the female sex (8).

#### PATHOLOGY OF THE GOITRE

*Macroscopically* the goitres are of glistening, fleshy appearance, bright or darkish red in colour, they stand out prominently against the surrounding tissues. They are of small size, being from 50 to 100 per cent larger than the normal organ. The isthmus is usually prominent, thickened and broadened. Commonly one lobe is more enlarged than the other, occasionally the enlargement is unilateral.

*Microscopically* the earliest evidence of change is seen in glands which are not appreciably enlarged but which appear bright coloured at post-mortem examination. More or less circumscribed areas (Plate XXVI, fig. 2)—commonly in the isthmus, the posterior border and the lower pole of one or other lobe—are seen to be depleted of colloid material or to be in process of depletion. The affected area assumes a cellular structure composed of a more or less solid mass of epithelium. This mass—the so-called intervesicular parenchyma—increases in bulk at the expense of the colloid-containing follicles appearing gradually to invade them and to crowd them out, some of these follicles contain larger or smaller numbers of free cells. Here and there the solid mass of epithelium is vesiculated by the collection among the cells of pools of new secretion (not colloid) (8). The cells subtending these pools present the typical features of secretory activity (Plate XXVII, figs. 6 and 7) rather deeply staining nuclei located at their base, and faintly basophil and granular cytoplasm. Passing from such non-goitrous glands to specimens of the actual goitres, one sees the more extensive crowding out of colloid-containing follicles (Plates XXVI and XXVII, figs. 3, 4 and 7) until in some sections there may be but two or three and occasionally none at all. Such colloid-containing follicles as exist are of normal appearance, the colloid deeply eosinophil, the acinar cells flattened or more commonly cuboidal, nuclei centrally placed and evenly distributed, and cytoplasm homogeneous and faintly acidophil. The whole or almost the whole organ is thus gradually depleted of its stored colloid and the normal structure of the gland is replaced by tissue in a state of intense

No.	Killed	135 to 195	100	122	122	60	F	421
No	Killed	08 to 195	100	117	118	45	M	422
No	Killed	07 to 195	100	101	103	48	F	423
No	Killed	11 to 195	100	114	114	40	M	424
No	Killed	08 to 195	100	83	84	46	F	425
No	Killed	06 to 195	100	89	96	33	M	426
No	Killed	06 to 195	100	92	92	41	M	415
No	Killed	04 to 195	100	92	94	47	F	416
No	Killed	07 to 195	100	102	102	53	M	417
No	Killed	06 to 195	100	82	86	49	M	418
No	Killed	11 to 195	100	83	83	50	F	419
No	Diarrhoea	08 to 195	81	55	62	32	F	420
Yes	Fits and Ophthalmia	065 to 195	83	48	62	36	F	409
No	Killed	065 to 195	99	90	94	49	M	410
No	Killed	04 to 195	99	74	92	52	M	411
No	Killed	08 to 195	99	98	98	55	F	412
No	Killed	10 to 195	99	91	94	48	M	413
No	Killed	03 to 195	99	77	82	32	F	414



XV	541	M	60	66	42	44	07 to 26	Paralysis	No
	542	F	57	57	35	44	11 to 26	Paralysis	No
	543	F	44	46	32	41	07 to 26	Paralysis	No
	544	M	47	55	37	46	08 to 26	Paralysis	Yes
	545	M	50	50	38	40	04 to 26	Asthma	No
	546	F	40	40	30	30	12 to 26	Asthma	Yes
XVI	397	M	52	110	110	104	12 to 195	Killed	No
	398	F	45	115	115	104	11 to 195	Killed	No
	399	F	50	113	113	104	195 to 195	Killed	No
	400	F	46	108	108	104	195 to 195	Killed	No
	401	M	46	121	121	104	195 to 195	Killed	No
	402	M	33	121	121	104	195 to 195	Killed	No



- (b) Those in which the protein-content is constant at 20 per cent and the white-flour-content is between 40 and 60 per cent, fats and salts being adequate, and the balance of carbohydrate being made up of purified starch (VIII to X)
- (c) Those in which the protein-content is constant at 20 per cent, fats and salts being adequate, while the white-flour-content is reduced to below 30 per cent and the balance of carbohydrate made up of purified starch (XI to XV)

The control diets (I and XVI) are not included in these three classes

Goitre occurred in the first and third class to an almost equal extent (38.9 and 33.3 per cent, respectively), but not at all in the second class, this is not to say that the diets of this class cannot cause goitre but that they did not cause appreciable enlargement of the gland in this experiment

A reference to Table I, in which the initial, final and maximum weights of each are given, will show that there were eight diets (I, II, III, IV, XII, XIII, XIV and XV) which failed to ensure even a moderate rate of growth. These may be excluded as unlikely to be of a composition comparable to any diets used by white-flour-eating peoples. They have, however, served a useful purpose in the experiment by indicating that insufficiency of vitamins of the B-class is a principal dietetic fault concerned in the production of this type of goitre. There are left seven diets (V, VI, VII, VIII, IX, X and XI) into which white-flour entered largely, these ensured a rate of growth which though not perfect was at least considerable. The following facts in regard to their association with goitre are to be noted —

- (1) Those containing 72 per cent or more of American white-flour and 15 per cent or less of protein, the fats and salts sufficing for normal needs, caused goitre in 33.3 per cent of young rats, despite the adequate ingestion of iodine (Table I)
- (2) Those containing 20 per cent of protein and between 40 and 60 per cent of American white-flour, the fats and salts sufficing for normal needs, did not cause goitre
- (3) One diet containing 20 per cent of protein and 30 per cent of American white-flour—the balance of carbohydrate being made up of purified starch and the fats and salts sufficing for normal needs—caused goitre in one out of six animals fed upon it, despite the adequate ingestion of iodine

The conclusion is thus reached that diets having the composition of those in groups 1 and 3 above will cause goitre in approximately 25 per cent of individuals consuming them despite the adequate ingestion of iodine. It will be noted from Table I that the amounts of potassium iodide ingested with these diets ranged between 0.4 and 2.6 mgrms per rat daily.

Among the sixteen experimental diets there were two (Nos. I and II) which caused goitre in four out of twelve animals, no iodine was added to them. The goitre arising in consequence of their consumption did not appear to differ histologically from those caused by the other diets. The further conclusion is,

goitre lie, therefore, in the differences in quality between the stock diet and the experimental diets. In comparison with the former the latter contained an insufficiency of accessory food factors belonging to the vitamin-B-class,\* they were also deficient in vitamin-C, in vitamin-A, in manganese, and in vegetable juices and roughage (cellulose and the indigestible parts of plants), while they were rich in vitamin-poor carbohydrate. Although it seems probable from the results of the experiment (Table II) that the insufficient provision of vitamins of the B-class was the principal dietetic cause of this type of goitre yet I am reluctant to attribute it wholly to this cause. For the present it is enough to say that it is prone to arise when green vegetable foods and fruit are excluded from a diet containing more than 60 per cent of white-flour, less than 20 per cent of protein, and fats and salts (including iodine) in adequate amounts.

There is another aspect of the causation of this type of goitre. All animals in each group were fed alike. There would seem at first sight to be no more reason, on dietetic grounds alone, that one or two animals in any one group should become goitrous than that the remainder should not. It is true that among the non-goitrous remainder there were some whose thyroids presented changes *in part of the gland* similar to those found in the goitrous organs, but there were others whose thyroids were to all appearances normal. Nothing, however, is more striking in the pathogenesis of deficiency disease than the variability of the effects of faulty food on different individuals and on different organs and tissues of the body. In one the nerves, in another the heart, in a third the gastro-intestinal tract may be so injured as to dominate the clinical scene. So it may be in the present instance the faulty diets used in these experiments, may have induced in some thyroids a graver state of physiological inefficiency than in others, and the changes in the goitrous glands may represent the attempt made by the physiological sub-normal gland to attain to a state of compensation. In this attempt it must inevitably fail since its malnutrition renders it inefficient as a machine.

On the other hand, the faulty diet *per se* may not have been the essential cause of the disease but have favoured the action on an ill-nourished and physiologically sub-normal gland of some goitrogenous agent peculiar to the animals which developed goitre. The diets with which the goitres were associated gave rise to a physiologically sub-normal, and sometimes to a pathological, state of the gastro-intestinal tract, and it seems probable that the exciting cause of the disease may have been of intestinal origin and of toxæmic nature. I have previously (8) postulated the existence of such 'toxæmic goitres' in constipated and imperfectly nourished girls of the working-classes. The present paper suggests at once the cause of the intestinal stasis (insufficiency of vitamin-B) (4) and of the 'toxæmic goitre' which may accompany it. While the cure of simple goitre which may so rapidly follow the removal of the stasis (8), without change in the state of nutrition of the subject, lends support to the view that the thyroid enlargement is in such cases of toxæmic origin. It may be, again, that faulty diets such as

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\* Experiments now in progress appear to indicate that there are two fractions in vitamin-B: a growth-promoting fraction and an anti-neuritic fraction, white-flour is not devoid of the latter, but is very deficient in the former, polished rice is very deficient in the latter—R. McC.



Fig 1—Normal thyroid gland of young rat same magnification as Figs 2, 3 and 4



Fig 2—Non-goitrous thyroid gland of rat No 510 fed on Diet VI, killed on 165th day of the experiment

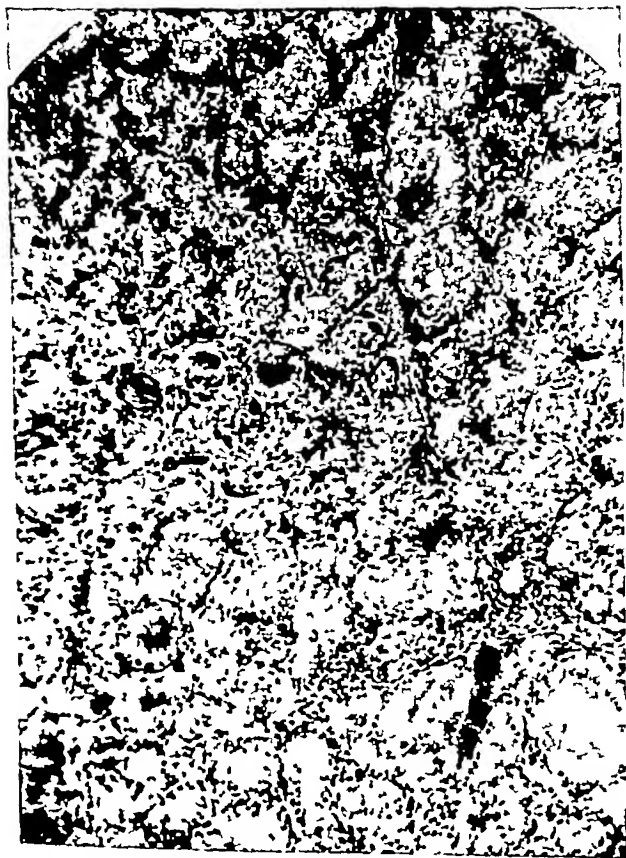


Fig 3—Goitrous thyroid gland of rat No 544 fed on Diet XV, died on the 46th day of experiment.



Fig 4—Goitrous thyroid gland of rat No 506 fed on Diet VI, killed on the 165th day of the experiment



be doubted that the pathological features of the 'endemic goitres' will be influenced by the food habits of residents in endemic zones where white-flour is the staple article of diet and milk and green vegetable foods are scarce, endemic influences may be expected to give rise to a pathological picture in the thyroid gland which will differ in detail from that resulting in localities where whole wheat-flour is used and milk and vegetable foods are abundant

### SUMMARY

(1) A new type of goitre has been produced, under experimental conditions in young rats, which is unrelated in its origin to iodine-deficiency

(2) It is to be distinguished ætiologically, pathologically and epidemiologically from the chronic hypertrophic or adeno-parenchymatous type of endemic goitre and from the diffuse colloid type of endemic goitre

(3) The basal factor in its causation is a diet containing more than 60 per cent of white-flour or of vitamin-poor carbohydrate, 20 per cent or less of protein with fats and inorganic salts (including iodine) in adequate amounts but no green vegetables nor fruit

(4) The goitre arising in these circumstances is characterized by intense secretory hypertrophy which ultimately leads to exhaustion of more or less of the epithelia and its replacement by non-secretory elements and fibrous tissue. These changes may be present without any marked increase in size of the gland

(5) It seems likely that this type of goitre will be found to occur sporadically amongst white-flour-eating peoples, that the subjects of its progressive stage will be prone to develop Graves's Disease, following such influences as fright, mental worry, pregnancy, lactation and attacks of acute infectious disease, and that the subjects of its retrogressive stage will exhibit greater or lesser degrees of myxœdema

(6) If this type of goitre be found to prevail sporadically in white-flour-eating peoples the additional provision of iodine in the food will not prevent it, but a well-balanced vitamin-rich diet will

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- |                          |  |
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| (6) McCARRISON (1918-19) | <i>Ind Jour Med Res</i> , VI, No 3, 275, No 4, 550, VII, No 2, 308           |
| (7) KORENCHESKY (1923)   | <i>Jour Path and Bact</i> , XXVI, No 3, 389                                  |
| (8) McCARRISON (1917)    | <i>The Thyroid Gland in Health and Disease</i> , London                      |



Fig 5—Normal thyroid gland of young rat same magnification as Figs 6, 7 and 8



Fig 6—Non-goitrous thyroid gland of rat No 510 same as Fig 2 higher magnification



Fig 7—Goitrous thyroid gland of rat No 506, same as Fig 4 higher magnification



Fig 8—Goitrous thyroid gland of rat No 529 fed on Diet II, died on the 127th day of the experiment



# PLATE XXVIII



Fig 9—Normal thyroid gland of young rat actively secreting phase same magnification as Figs 10, 11 and 12



Fig 10—Goitrous thyroid gland of rat No 515 fed on Diet V died on the 94th day of the experiment.



Fig 11—Goitrous thyroid gland of rat No 514 fed on Diet V died on the 75th day of the experiment



Fig 12—Goitrous thyroid gland of rat No 409 fed on Diet XI died on the 83rd day of the experiment

been subjected to a certain amount of adverse criticism in India,\* and, since it is not meet that judgment should be allowed to go by default, it is necessary that an attempt should be made to justify the preparation of these forecasts at the bar of scientific opinion. It is, therefore, proposed to detail the result of the analysis of the malaria forecast for the year 1926 and thereafter to describe the precise mode of its preparation and to appraise its accuracy.

## II THE MALARIA FORECAST FOR THE YEAR 1926

The malaria forecast which was prepared during the absence of the writer in England, by the Director of Public Health, Punjab, was in the following terms —

### *Punjab Public Health Department*

#### *Malaria Forecast based upon data up to August 23rd, 1926*

Speaking generally conditions are favourable for an outbreak of malaria in the eastern and northern districts, but detailed figures are only available for the following districts listed in order of probability of the height of incidence —

- (1) (a) Hissar, (b) Rohtak, (c) Gurgaon, (d) Jullundur,  
(e) Ludhiana
- (2) Rawalpindi
- (3) Karnal
- (4) Sialkot
- (5) Amritsar
- (6) Ambala

In the southern and western districts conditions generally are unfavourable for an outbreak of malaria, but detailed figures are only available for the following districts which may be expected to have an incidence approximately normal or below normal —

- (1) Muzaffargarh
- (2) Dera Ghazi Khan
- (3) Mianwali
- (4) Lahore
- (5) Multan
- (6) Montgomery

*Simla, August 31st, 1926*

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\* NOTE—*The Statesman* (of Calcutta), dated September 9th, 1924, contained the following editorial comment 'It is both unusual and wrong for Health Authorities to issue forecasts regarding the occurrence of epidemics as if they were crops or the monsoon and the Punjab Health Department would be well advised not to follow up its preliminary forecast as to the incidence of malaria in the Province. A well-marked epidemic focus, we read, is likely to develop in the districts of Lahore, Amritsar and the east of the Montgomery district. The sturdy Jat peasantry of the *tahsils* indicated will possibly not be alarmed by the forebodings of a Simla official, but the principle of the forecast is bad, sickness being as much psychological as physiological'—C. A. G.

those used in these experiments give rise to different biochemical disturbances in different individuals with the production in some of abnormal metabolites which excite the thyroid unduly or deplete it of the hormone necessary for their combustion

There are thus three sites at which the essential cause of this type of goitre is to be sought. The first in the gland itself, where inefficiency of the thyroid machine may be brought about by its malnutrition with impediment to the normal revolution of its cycle of activity, the second in the body fluids and tissues where imperfect metabolism may be assumed to result in the production of abnormal metabolites and to cause depletion of the thyroid hormone necessary for their combustion, and the third, in a physiologically sub-normal or pathological alimentary tract whence bacterial agents or their toxins may find their way into the circulation and induce pathological changes in the thyroid gland. But at all three sites the common factor is imperfect nutrition induced by faulty food, the general statement I have already made as to the dietetic cause of this type of goitre will serve until we know more of the part played by the thyroid gland in the animal economy, and of the biochemical phenomena associated with faulty nutrition

#### COMMENTARY

In view of the confusion which exists in regard to the causation and prevention of the thyroid diseases included under the generic term 'simple goitre' the results of these experiments are illuminating. If, as seems likely, the type of goitre here described be found to occur sporadically amongst white-flour-eating peoples then the view that *all* 'simple goitres' are due to iodine-deficiency and preventable by the prophylactic use of iodine must be abandoned. The relation of iodine to each type of 'simple goitre' must be definitely defined, this has been attempted, as shown in the previous paper (page 207) for the classical type endemic goitre—the chronic hypertrophic or parenchymatous type, it remains to be done for the diffuse colloid type of endemic goitre

The experimental evidence here provided makes it probable that in Western countries this new type of goitre will be encountered in its progressive stage in childhood and in young women whose food contains much vitamin-poor carbohydrate, little suitable protein and less green vegetables and fruit, and in its retrogressive stage in older subjects whose food has similar faults. If this should prove to be so iodine will neither prevent nor cure the disease, but a well-balanced diet rich in vitamins will. These matters can, however, only be decided by clinical and epidemiological observation, and by 'goitre-surveys' which take into consideration the food habits of the people and are not limited to one section of the community—the children. Outside the laboratory it will probably be found that the purity in type of this variety of goitre may commonly be obscured by the interaction, with the nutritional factors which give rise to it, of other goitrogenous influences, such as the ingestion of an excess of lime or of fats, and insanitary conditions of life both intrinsic and extrinsic to the individual. Such influences as these may be expected to distort its characteristic histo-pathological picture and give variety to the characters of the goitres met with in different individuals or in different localities. Further, it can hardly

TABLE I

*The Epidemic Figures of Districts, 1926*

E F under 2 0		E F 2 0—3 0		E F 3 0—4 0		E.F over 4 0
* Dera Ghazi Khan	1 00	<i>Ambala</i>	2 00	<i>Amritsar</i>	3 25	<i>Sialkot</i> 4 25
* Mianwali	1 00	Sheikhupura	2 00	Gurdaspur	3 45	
* Muzaffargarh	1 10	Attock	2 00			
* Multan	1 10	<i>Ludhiana</i>	2 00			
Jhang	1 10	* Lahore	2 10			
Lyallpur	1 10	Gujranwala	2 30			
* Montgomery	1 15	Hoshiarpur	2 30			
Shahpur	1 20	Jhelum	2 45			
Ferozepur —	1 40	<i>Gurgaon</i>	2 50			
<i>Karnal</i>	1 40	<i>Rohtak</i>	2 50			
Kangra	1 50	Gujrat	2 60			
<i>Rawalpindi</i>	1 90	<i>Jullundur</i>	2 70			
		<i>Hissar</i>	2 80			

NOTE.—The ten districts shown in italics are those in which an epidemic of malaria was predicted to occur. The six districts marked with an asterisk are those in which the incidence of malaria was expected to be normal, whilst, owing to absence of data, the forecast made no reference to the remaining twelve districts.

A more pronounced measure of success attended the prediction upon its negative side—and from the scientific and practical standpoint a negative is as valuable as a positive prediction—for it will be seen that in five out of the six districts in which the incidence of malaria was anticipated to remain normal the prediction was borne out by the event. These five districts are thus congregated, as they should be, at the top of the first column of Table I, but, here again, no

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# THE FORECASTING OF MALARIA EPIDEMICS WITH SPECIAL REFERENCE TO THE MALARIA FORECAST FOR THE YEAR 1926

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## I INTRODUCTION

THE first tentative forecast in regard to the occurrence of epidemic malaria in the Punjab was prepared in the year 1921 and an account of this prediction together with the result of a critical examination of its accuracy was subsequently published in the *Indian Journal of Medical Research* (1)

Emboldened by the success achieved on this occasion malaria forecasts were prepared for the years 1922 and 1923 and the result of the subsequent analysis of these forecasts was again published in the *Indian Journal of Medical Research* (2) (3)

The accuracy consistently displayed by these three forecasts—and, it may be added, by the forecasts for the years 1924 and 1925—renders it permissible to conclude that the time has arrived when the implications attaching to the apparent facility with which accurate malaria forecasts can be prepared should be accorded explicit recognition. In particular, it seems proper, since the method of forecasting can no longer be regarded as in the experimental stage, that a detailed account of their mode of preparation should be placed upon record.

The expediency of so doing is enhanced by certain other considerations. The published account of the analyses of the forecasts for the years 1921, 1922 and 1923 has attracted the attention of malariologists in Europe and America and their encouraging comments suggest that a description of the method of forecasting would be welcomed. On the other hand, the principle as well as the practice of issuing predictions regarding the occurrence of these epidemics has

TABLE II

*The Epidemic Figures of Registration Circles*

District	Number of Registration Circles with an Epidemic Figure of —				Diffusion Index	Intensity Index
	1 0—2 5	2 5—5 0	5 0—7 0	7 0—10 0		
Hissar	11	13	2	0	58	8
Rohtak	11	9	0	0	82	0
Gurgaon	10	14	0	0	58	0
Karnal	23	2	0	0	8	0
Ambala	12	6	1	0	37	5
Kangra	16	0	0	0	0	0
Hoshiarpur	8	15	0	0	65	0
Jullundur	12	6	0	0	33	0
Ludhiana	10	4	0	0	40	0
Ferozepur	25	0	0	0	0	0
Lahore	24	4	1	0	17	3
Amritsar	4	9	2	0	73	13
Gurdaspur	3	17	1	0	86	5
Sialkot	6	14	6	2	78	28
Gujranwala	9	10	0	0	52	0
Sheikhupura	18	3	0	0	14	0
Gujrat	6	9	2	0	64	12
Shahpur	19	4	0	0	17	0
Jhelum	7	8	0	0	53	0
Rawalpindi	10	4	0	0	28	0
Attock	11	4	0	0	27	0
Mianwali	16	0	0	0	0	0
Montgomery	24	0	0	0	8	0
Lyallpur	21	0	0	0	0	0
Jhang	15	0	0	0	0	0
Multan	21	0	0	0	0	0
Muzaffargarh	24	0	0	0	0	0
Dera Ghazi Khan	23	0	0	0	0	0

### III THE ANALYSIS OF THE FORECAST

It will be recollected that the forecast, which is usually prepared and issued on the 1st of September, refers to an epidemic which takes place in the months of October and November. It will also be recalled that the distribution and intensity of epidemic malaria in different parts of the province can be accurately determined by calculating the 'epidemic figure' (E F) which represents the number of times the 'fever' mortality in the month of October exceeds the mean monthly 'fever' mortality of the four months from April to July of the same year. It is thus possible to calculate the epidemic figure of the province as a whole, of each of the 29 districts and of each of the 574 registration circles.

#### (a) *The Provincial Epidemic Figure*

The provincial epidemic figure merely indicates the general intensity of the epidemic throughout the province. In a year when a major epidemic occurs this figure may be as high as 6.0—10.0, in years in which malaria does not become epidemic it does not exceed 1.0, but in many years a mild epidemic of restricted distribution is associated with an epidemic figure of 1.1—1.5. In the year 1926 the provincial epidemic figure was 1.99, in other words the mortality caused by epidemic malaria during the month of October was approximately double the normal. This figure therefore suggests the occurrence of a distinct epidemic of moderate intensity and it is incompatible with the occurrence of a severe and widespread epidemic. The forecast which stated that the epidemic would be restricted to certain districts in the east and north of the province was therefore justified by the event.

#### (b) *The District Epidemic Figures*

A minor epidemic may however be associated with the occurrence of an epidemic of considerable intensity in a restricted number of districts and it is therefore necessary to scrutinize the district epidemic figures. These figures are shown in Table I where they are ranged in four groups in order of magnitude of their respective epidemic indices.

A scrutiny of Table I discloses the fact that, on its positive side, the forecast achieved a fair degree of accuracy. Thus two out of the three districts (Sialkot and Amritsar), in which an epidemic of conspicuous intensity occurred, were mentioned in the forecast, whilst the third district (Gurdaspur), was one of the districts for which no data were available.

Of the remaining eight districts in which malaria was expected to assume epidemic proportions it will be seen that a distinct epidemic, which is represented by an epidemic figure of 2.0—3.0, actually took place. The only appreciable inaccuracy was therefore the prediction that an epidemic would occur in the districts of Rawalpindi and Karnal, but it will be noted that the epidemic figure of Rawalpindi district was 1.90. No data were unfortunately available in regard to the districts of Attock, Sheikhupura, Gujranwala, Hoshiarpur, Jhelum and Gujrat.

The economic factor is derived from data obtained from the Director of Land Records, Punjab, in regard to the average price of food-grains and it thus furnishes an index of the economic condition of the population. The significance attached to this factor is dependent upon the induction derived from statistical analysis that although famine and lesser degrees of economic stress are incapable of determining an epidemic, they exercise a profound influence, other factors being favourable, upon the magnitude and intensity of epidemics of malaria.

The data regarding the spleen-rate are obtained by means of a spleen census of school-children as the result of which some 40,000 scholars located in 286 representative localities have been examined twice a year (June and November) during the past twelve years. The significance attached to this factor is dependent upon the observation that epidemics of malaria of appreciable intensity never occur in localities in which the spleen-rate, as compared with the previous five years, is absolutely and relatively high. A study of these data enables fluctuations of the spleen-rate to be followed, and a rough inverse relationship, other things being equal, has been found to exist between the height of the spleen-rate during the month of June and the intensity of epidemic malaria in the following autumn.

The precise nature of the influence of atmospheric humidity in the mechanism of epidemic malaria cannot be fully detailed here and it must suffice to state, pending the publication of a complete account of the research, that atmospheric humidity, largely but not solely by reason of its influence upon the longevity of mosquitoes and upon their power to transmit infection, is an extremely important factor in determining an epidemic (5).

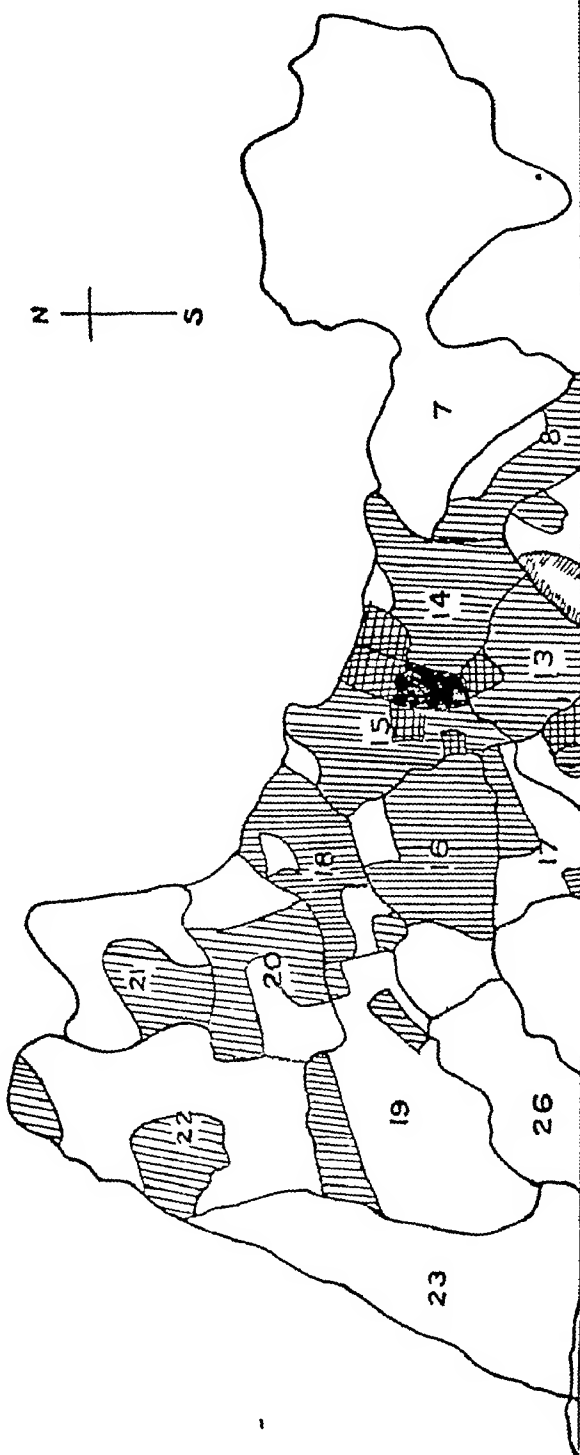
Unfortunately the humidity factor cannot be directly measured except in a few localities owing to the fact that there are only ten meteorological stations in the Punjab. It has, however, been shown by statistical analysis that atmospheric humidity is highly correlated with rainfall in the Punjab during the pre-epidemic period (the months of July and August) and as there are 192 rainfall recording stations in the province, the rainfall data of these stations have been utilized for the purpose of these forecasts as an index of atmospheric humidity.

It has been shown elsewhere (6), as the result of statistical analysis, that the combined July-August rainfall is more closely correlated with 'fever' mortality during the months of October and November than is the rainfall of any other months or combination of months and that in particular the rainfall of September exercises no appreciable influence in determining the occurrence of epidemic malaria and it thus comes about that the rainfall data required in connection with the preparation of a malaria forecast can be assembled on September 1st at the earliest or in other words about four to six weeks before the onset of these epidemics.

The rainfall data are obtained from the Director of Land Records, Punjab, to whom they are reported weekly, but it has hitherto only been possible to obtain on September 1st the data referring to the last week in August in the case of 14 representative districts. A preliminary forecast is therefore usually issued on the 1st of September and the final forecast about the twelfth of the month. The normal July-August rainfall of these 192 recording stations being known, it is

## MAP OF PRINCIPALITIES.

EPIDEMIC MALARIA, 1926



## DISTRICTS

1956114

## Kohtak

Gurğon

## Karnal

## Ambria

## Similar

Հանգր

**Hoshiarpur**

willundur

udhiana

**eropezur**

shore

**mrtsar**

TABLE III  
The Forecasting Factors, 1926

District	THE RAINFALL FACTOR	THE SPLEEN- RATE FACTOR	THE EPIDEMIC POTENTIAL FACTOR	The Epide- mic Figure, 1926
	(Departure from mean of July- August Rain- fall )	(The Spleen-rate in June, 1926 )	(The coefficient of Variability of 'Fevers' in October )	
Fissar	+6 94	14 0	67 5	2 80
Rohtak	+5 24	22 4	81 8	2 50
Gurgaon	+8 65	9 3	104 7	2 50
Karnal	+0 79	39 1	59 5	1 40
Ambala	+3 10	15 2	61 7	2 00
Kangra	+5 34	15 5	21 8	1 50
Hoshiarpur	+3 28	9 2	50 2	2 70
Jullundur	+5 67	2 9	66 6	2 30
Ludhiana	+4 37	6 9	88 8	2 00
Ferozepur	+2 99	7 3	62 1	1 40
Lahore	+5 25	9 3	84 4	2 10
Amritsar	+6 51	12 6	91 2	3 25
* Gurdaspur	+2 56	14 5	61 1	3 45
Sialkot	+6 57	9 9	78 9	4 25
* Gujranwala	—0 55	11 4	86 5	2 30
Sheikhupura	+3 23	10 4	86 5	2 00
* Gujrat	+1 79	9 7	107 6	2 60 <sup>1</sup>
Shahpur	+2 24	9 8	100 0	1 20
Jhelum	+6 48	11 0	82 1	2 45
Rawalpindi	+6 83	4 4	42 3	1 90
Attock	+6 37	12 5	37 5	2 00
Mianwali	—1 53	13 8	52 3	1 00
Montgomery	+3 16	9 5	112 5	1 15
Lyallpur	+1 88	10 5	69 2	1 10
Jhang	—1 20	8 3	78 1	1 10
Multan	+0 07	13 6	76 9	1 10
Muzaffargarh	—0 93	37 9	45 4	1 10
Dera Ghazi Khan	—0 82	13 0	69 2	1 00

NOTE.—The districts marked with an asterisk are those in which the forecast based upon the data contained in the Table was appreciably inaccurate.

data were available in the case of the other districts mentioned in this list (Jhang, Lyallpur, Shahpur, Ferozepur and Kangra)

The sole error on the negative side of the forecast was in classifying Lahore district, which experienced an epidemic whose intensity is represented by an epidemic figure of 2.10, amongst those districts in which the incidence of malaria would remain approximately normal

A consideration of the district epidemic figures therefore suggests that the forecast, apart from omissions due to the absence of data, was approximately accurate in the case of fourteen out of sixteen districts, the two appreciable inaccuracies having reference to Karnal and Lahore districts

### (c) *The Epidemic Figures of Registration Circles*

There are 574 registration circles in the Punjab or an average of about 20 in each district and it thus comes about that a relatively low district epidemic figure is not incompatible with the occurrence of an intense epidemic in a small number of registration circles. It is, therefore, necessary to examine the epidemic figures of these circles and, with their aid, to plot out on a map (Map I) the precise distribution of the epidemic. A summary of these figures is given in Table II to which has been added a 'diffusion index' and an 'intensity index,' the former representing the number of registration circles with an epidemic figure of over 2.5 expressed as a percentage of the total number of registration circles in the district, whilst the intensity index refers to the percentage of registration circles in each district with an epidemic figure of 5.0 or more

A scrutiny of the figures given in Table II and of Map I shows that the distribution and intensity of epidemic malaria during the autumn of the year 1926 exhibited the following features —

- (1) In the extreme south of the province an epidemic of malaria occurred in the districts of Hissar, Rohtak and Gurgaon
- (2) In the east the districts of Jullundur, Ludhiana, Hoshiarpur and Ambala were extensively involved
- (3) The main feature of the epidemic was the occurrence of a compact epidemic focus, with its 'nucleus' in Sialkot district, in the districts of Sialkot, Gurdaspur, Amritsar, Lahore, Gujranwala and Gujrat
- (4) To the north of the Jhelum river a diffuse epidemic focus of mild intensity prevailed in the districts of Jhelum, Rawalpindi and Attock
- (5) In the remaining districts of the province, including all the districts in the west and south-west, the incidence of malaria was normal
- (6) So far as *diffusion* was concerned the most widely affected districts were Gurdaspur, (86), Rohtak, (82), Sialkot, (78), Amritsar (73), Hoshiarpur, (65), Gujrat, (64), Hissar and Gurgaon, (58), Jhelum, (53) and Gujranwala, (52)
- (7) In respect of *intensity* the epidemic was everywhere mild except in the districts of Sialkot, (28), Amritsar, (13), Gujrat, (12), Hissar, (8), Ambala and Gurdaspur, (5) and Lahore, (3).



because they permit of the inspiring hope that a quest embarked upon some 14 years ago is approaching its objective

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## IV THE METHOD OF FORECASTING

It will be seen, apart from omissions due to the absence of data, the distribution of the epidemic was in close conformity with the terms of the forecast prepared on August 31st, 1926, but, as the omissions were rather numerous and certain errors require to be explained, it is necessary, in the light of the complete data, to examine the method of forecasting with a view to determining whether the defects brought to light are dependent upon errors of a systematic or asystematic order

The method of forecasting is based upon the view put forward as a working hypothesis in the year 1914 (4) that malaria epidemics are the outcome of 'loss of equilibrium' between 'infection' and 'immunity'. A detailed account of the investigation as the result of which this working hypothesis has been raised to the rank of a theory will shortly be published, and it will, therefore, suffice here to state that if this theory does resume a fundamental truth—and these forecasts constitute one of the various methods that have been devised to test the accuracy of the theory—it should be possible, if suitable factors representative of 'infection' and 'immunity' can be assembled before the event, to forecast the occurrence of these epidemics with mathematical precision

In the light of this view the following five factors have been selected as of epidemiological significance in the preparation of malaria forecasts —

- (1) The humidity factor of which the July-August rainfall of 192 rainfall recording stations is the index
- (2) The spleen-rate factor as determined by the spleen-rate of some 286 representative localities during the previous five years
- (3) The economic factor of which the average price of food-grains in the Punjab during the previous five years is the index
- (4) The 'epidemic potential' factor of which the coefficient of variability of 'fevers' during the month of October for the period 1868—1921 is the index
- (5) The anopheline factor as determined by the total prevalence of natural 'carriers' during the months of July and August

As regards the anopheline factor, observations having appeared to show that no significant difference could be detected from year to year in the total number of anophelines present in the epidemic area during the months of July and August, this factor, to which at one time importance was attached, has latterly been ignored in the preparation of malaria forecasts. The 'epidemic potential' factor, which is obtained by multiplying the standard deviation of 'fevers' during the month of October by 100 and by dividing the product by the number of observations (55 years), indicates the relative liability of each district to epidemic visitations and the significance attached to it is illustrated by the fact that in the preparation of these forecasts no epidemic will be expected to occur, however favourable the other factors may be, in any district with an extremely low coefficient of variability of 'fevers'.

tertian malaria a clinical cure was recorded in 66·6 per cent, but only 33 per cent were parasite-free

Fifty-four of the patients were observed for periods of 2 to 15 months after the end of treatment, and of forty cases of benign tertian malaria so observed 29 (72 per cent) had not relapsed, but 8 of these, or 20 per cent of the total cases, still harboured parasites, making a total of 48 per cent relapse, according to the standard used in our work (Sinton, 1926)

Of 6 cases of malignant tertian malaria so observed two had relapsed and one other had parasites in the peripheral blood, while out of 8 cases of mixed infection two had relapsed and two others still harboured parasites. This makes a total of 54 patients observed of whom 27·7 per cent had already relapsed and another 20·4 per cent had parasites in the peripheral blood at the time of examination, i.e., a total of 48 per cent of relapses

Fletcher (1925) tried the effects of this drug on malarial patients in the Malay States, and as a result of his clinical tests states that it had no effect on the malaria parasites. Schrumph-Pierron and Pagnier (1925) say that they got the impression from the use of the drug that its results were superior to quinine in chronic cases of malaria, but they recommended further work on the subject

#### METHODS AND TREATMENT

The Haco Company in their circulars state that '6 to 8 pills a day are generally sufficient. Here experience has shown that when there has been previous fever, or fever induced by the exhibition of Peracrina, an increased dose of 10 to 12 pills will speedily reduce the temperature. It is indicated, therefore, that patients who have had fever before treatment should start with large doses of 10 to 12 pills a day, and one should only administer the smaller doses of 6 to 8 pills when fever has abated. In these cases of malaria, where the attacks are infrequent but where the general state of the health shows that the biological equilibrium between the disease and resisting power is gradually decreasing, small doses from the beginning are indicated. Should, however, attacks with high temperature occur, the daily dose is to be increased accordingly.' 'So far, Peracrina 303 has proved efficacious mainly in the treatment of chronic malaria.' It is also recommended that the treatment should be continued until the peripheral blood is free of parasites for at least 15 days.

The ten patients treated by us were young adult British soldiers suffering from benign tertian malaria, as diagnosed by microscopical examinations of the blood. The cases were mostly of a chronic nature (*see below, medical histories*)

The treatment given was as follows — For the first 3 days, 6 pills daily, from the 4th to 7th days, 8 pills daily, from the 8th to 10th days, 9 pills daily, and from the 11th day onward, 12 pills daily until the end of treatment. In Case 1 from the 66th to 114th days, 15 pills were given daily, and, in Case 2 from the 25th to 108th days a similar dosage was given.

only necessary to obtain the 'actuals' for the year in question and to calculate the departure from the mean in order to obtain the requisite rainfall factor

It will thus be seen that the forecasting factors may be divided into two groups, one of which contains the humidity factor, and the anopheline factor, which are designed to provide early information in regard to the amount of 'infection,' and the other group comprising the spleen-rate factor and the economic factor, which are calculated to afford a rough index of communal 'immunity'

The complete data up to September 1st, 1926, now being available, the manner in which these data are utilized in the preparation of a malaria forecast may conveniently be illustrated in reference to the forecast for the year 1926

For reasons already indicated the anopheline factor will be ignored and, in the case of the year 1926, it is unnecessary to consider in detail the economic factor except to remark, as the result of a series of favourable monsoons, that 'prices' were everywhere relatively low as compared with the past quinquennium and the economic factor was consequently everywhere unfavourable to the occurrence of an epidemic of high intensity

The three important factors are therefore the rainfall factor, the spleen-rate factor and the 'epidemic potential' factor and the data regarding them, which were available on September 1st, 1926, are summarized in Table III to which has been added in the last column the actual incidence of epidemic malaria in each district as determined by the epidemic figure for the month of October, 1926

A scrutiny of the data contained in the first three columns of Table III justifies the following inferences in regard to the incidence of epidemic malaria in the Punjab during the months of October and November, 1926

All the factors are favourable to the occurrence of an epidemic of moderate intensity in the districts of Hissar, Gurgaon and Rohtak. No epidemic would be expected to occur in Karnal district owing to the fact that the rainfall factor and the spleen-rate factor are both unfavourable and the inclusion of this district in the list of those in which an epidemic was predicted to occur was therefore not justified

In Kangra district the rainfall factor was favourable but the low epidemic potential factor (21.8) is inconsistent with the occurrence of an epidemic of appreciable intensity in this district

All three factors are favourable to the occurrence of epidemic malaria in the districts of Jullundur, Ludhiana and Hoshiarpur and an epidemic of some intensity is likely to occur in the first-named of these districts. An epidemic focus would be expected to occur in the districts of Sialkot, Amritsar and Lahore and, in view of the favourable nature of the factors, to attain relatively high intensity in Sialkot and Amritsar districts. The inclusion of Lahore district in the list of districts in which the incidence of malaria was expected to remain normal, was not in accordance with the forecasting data

A diffuse epidemic is likely to prevail in the districts of Jhelum, Rawalpindi and Attock, but in view of the low epidemic potential factor of the two last-named districts, it is probable that the epidemic will be of mild intensity in these districts

## Case No 2—J S, aged 23 years

This patient had a history of 1 previous relapse

*Treatment*—This was carried out as described above and continued until the 108th day, during which time he received 1,308 Peracrina pills. From the 66th day until the end of treatment the dose given was 15 pills daily. No quinine was given.

*Temperature*—This never rose above normal during the whole period of treatment.

*Parasites*—1st + + + +, 2nd +—, 3rd + + + +, 4th +—, 6th +—, 8th +—, 10th +—, 13th to 21st +—; 33rd and 34th +—, 38th and 39th +—, 42nd +—, 56th +—, 66th +—, 72nd and 73rd +—, 89th and 90th +—, 98th and 99th +—, 106th +—

*Spleen*—*Vide* Table I

*Result*—No relapse recorded

## Case No 3—S G, aged 23 years

This patient had a history of three previous attacks

*Treatment*—This was as described above and continued until the 56th day, during which time 537 pills had been taken. He also received 20 grains of quinine in solution during the 4th day on account of the severity of his symptoms.

*Temperature*—Rigors with a temperature occurred on the 1st and 3rd days of treatment with temperatures of 104°F. After this time, the temperature remained normal until the 17th day when it rose to 99.5°F. On the 20th day it again rose to the same level and on the 21st day a rigor with a temperature of 101°F was recorded. No other rise of temperature was recorded during treatment.

*Parasites*—1st + + + +, 3rd + + +, 4th + + + +, 5th +—, 6th +, 17th to 19th +—, 20th +, 21st + +, 22nd +— No parasites were found during the remaining 34 days of treatment.

*Spleen*—*Vide* Table I

*Result*—The patient relapsed parasitically during the 5th week of observation after treatment. He was given a course of 28 days' treatment with 'Malarene' (standardised cinchona febrifuge), after which he was observed for 8 weeks without a relapse.

## Case No 4—J M, aged 25 years

The patient had a history of three previous attacks of malaria

*Treatment*—The patient received 945 pills of Peracrina during a period of 95 days. He was given quinine in solution on three occasions during treatment, a total of 70 grains.

The further details of this case are shown on Chart I.

*Result*—No relapse was recorded during observation.

## Case No 5—G M, aged 23 years

The patient had a history of four previous attacks of malaria

*Treatment*—He received 465 pills during the 45 days of treatment.

*Temperature*—No rise of temperature to 99.5°F or over was recorded during treatment.

Finally, the incidence of malaria would be expected to remain approximately normal in the districts of Karnal, Kangra, Gujrat, Gujranwala, and in all the districts in the west and the south-west of the province

When the actual incidence of epidemic malaria, as given in the last column of Table III, is compared with the above statement it is clear that the correspondence between anticipations based upon the forecasting data and actual happenings is extremely close

The two discrepancies in the original forecast are found to be due to errors of an unsystematic order but a study of the complete data has brought to light the fact that in three districts—none of which were mentioned in the original forecast owing to absence of data—the incidence of epidemic malaria was not in accordance with anticipations

It is scarcely to be expected that a forecast based upon data which necessarily constitute random samples should attain absolute accuracy, but if the theory upon which the method of forecasting is based be correct, it is necessary to consider whether any explanation can be given of the fact that in the districts of Gujrat, Gujranwala and Gurdaspur an epidemic of malaria of appreciable intensity was associated with a rainfall factor definitely unfavourable to the occurrence of an epidemic

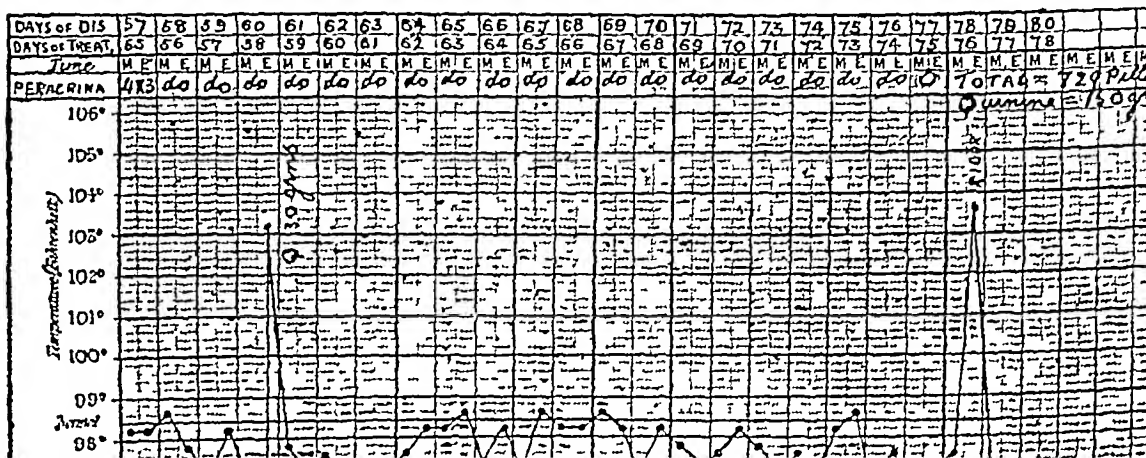
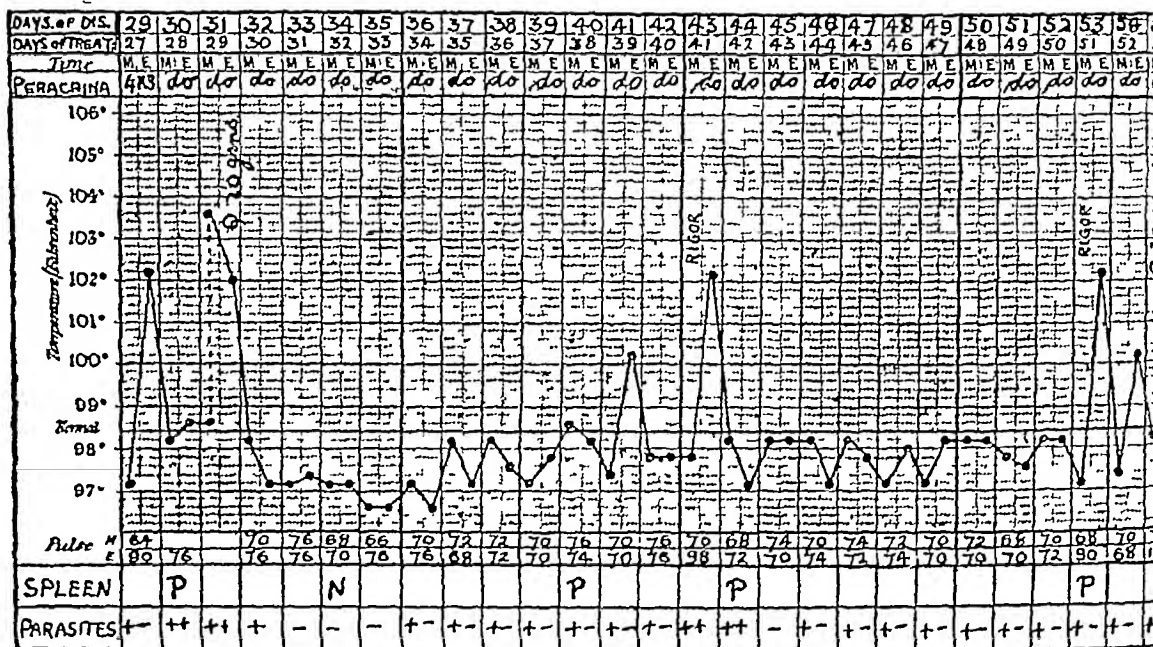
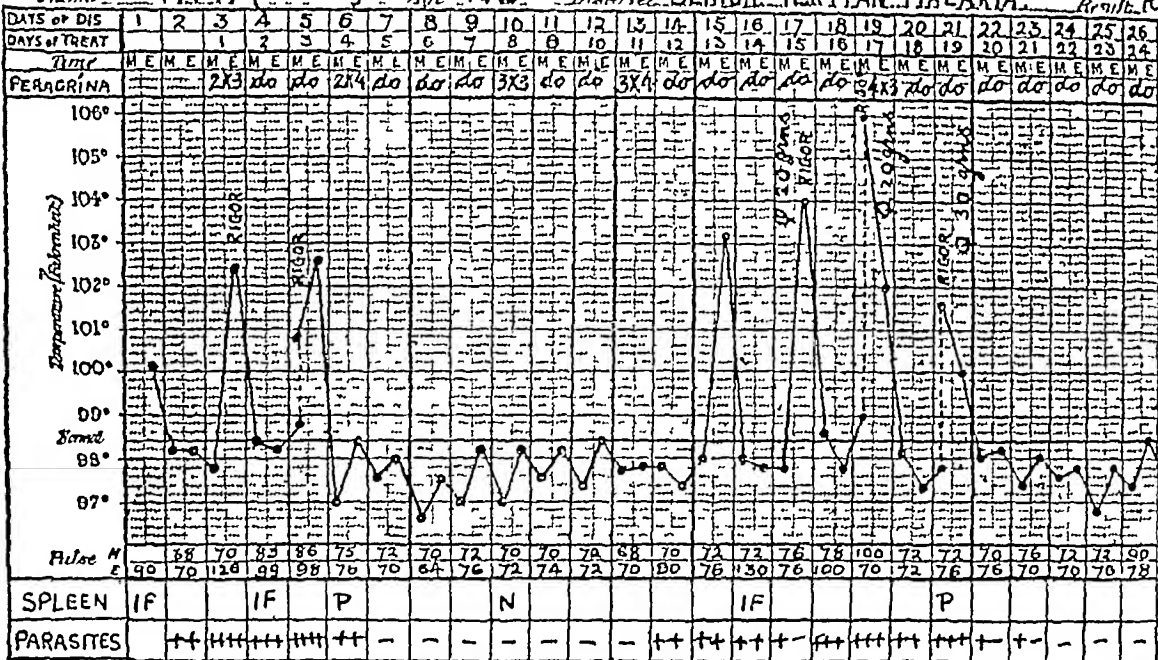
It will be observed from a scrutiny of Map I that these three districts are all located in close proximity to the montane tract and they are known to be peculiarly liable to inundation during the monsoon as the result of heavy rainfall in the hills. In these circumstances it is possible that the correlation between atmospheric humidity and *local* precipitation is less close in the sub-montane districts than it is in districts where atmospheric humidity is mainly a reflection of local rainfall. Whether this surmise be correct or no—and steps are being taken to verify its accuracy—it is significant that such errors as have been discovered in the forecasts during the past six years have mainly occurred in connexion with the sub-montane districts. These minor errors do not however materially detract from the general accuracy of the forecast and, although it is necessary that they should, if possible, be eliminated, there is some reason to believe that they are the outcome of imperfections of the data rather than the result of the inadequacy of the theory upon which the method of forecasting is based

## V CONCLUSION

Six successive forecasts have now been prepared, the result of an analysis of four has been published and each forecast has exhibited conspicuous accuracy

The method of forecasting and the mode of preparation of an actual forecast have now been described and thus the relevant facts for the formation of a considered judgment are available. To some it has appeared that the publication of these forecasts serves no useful purpose, others have considered the prediction of epidemics is 'wrong' in principle and they have not hesitated to adopt the view that the cause of these phenomena are 'as much psychological as physiological'. To the writer it would appear that predictions which give a month's warning of the occurrence of an impending catastrophe are of considerable value, not only because they enable timely action to be taken to mitigate misery and distress, but

Brute R



# STUDIES IN MALARIA WITH SPECIAL REFERENCE TO TREATMENT

## Part VI.

### THE TREATMENT OF BENIGN TERTIAN MALARIA WITH PERACRINA 303

BY

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*(Research carried out under the Indian Research Fund Association)*

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WHILE working with the Swiss Red Cross Expedition in the famine districts of Russia, Walker (1924) conducted a series of experiments on the treatment of malaria by means of two new drugs—Peracrina 303 and Peracrina 404. As a result of his work he claims that the former drug is a specific remedy against malaria, well suited to replace or supplement quinine in many cases, while the latter drug, although a remedy for this disease, appears to be liable to give rise to dangerous effects in some cases.

These drugs are manufactured by the Haco Company, Ltd, of Berne. Peracrina 303 is said to consist of specific albuminates combined with tryptaflavine, the compound containing 10 per cent of the latter drug. It is made up in pills of 0.5 grm each, i.e., 0.05 grm tryptaflavine in each pill. Peracrina 404 is stated to be an iodated compound of tryptaflavine and albumin.

Walker (1924) summarises his results as follows—(a) out of 73 cases of benign tertian malaria, 71.2 per cent were cured clinically, but in only 56.2 per cent of the total number was the peripheral blood free of parasites when the patients were discharged, (b) out of 20 cases of malignant tertian malaria 65 per cent were cured clinically, but only 25 per cent were parasite-free on discharge, and (c) of 21 mixed infections of benign and malignant



*Parasites*—1st and 2nd +++ , 3rd ++ , 4th and 5th +—, 13th +—, 15th and 16th ++ , 17th +++ , 36th +—, 53rd to 55th +—, 57th +—; 59th to 61st +—, 63rd +—, 72nd +—, 74th +—

*Spleen*—*Vide* Table I

*Result*—No relapse was recorded during observation

Case No 8—W P, aged 23 years

The patient had a history of 15 or 16 previous relapses

*Treatment*—The patient received 729 pills during the 74 days of treatment. He was also given quinine on six occasions during treatment, a total of 150 grains (*vide* Chart II)

*Temperature and parasites*—*Vide* Chart II. The blood of this patient was never negative for 15 consecutive days during treatment

*Spleen*—*Vide* Table I

*Result*—The patient relapsed during the first week of observation following treatment. He was then given four different courses of treatment, but relapsed after each treatment

Case No 9—J M, aged 31 years

The patient had a history of 10 previous attacks of malaria

*Treatment*—The patient received 717 pills of Peracrina during the 72 days of treatment. Twenty grains of quinine were given on two occasions during treatment

*Temperature*—Rigors occurred on the day before treatment was started and on the 1st and 2nd days of treatment with temperatures of 101° to 102°F. For this reason 20 grains of quinine were given, after which no rise of temperature was recorded until the 20th day when a rigor again occurred, and another 20 grains of quinine were administered

The temperature rose to 100°F on the 23rd day and remained normal for the rest of the treatment

*Parasites*—1st +++ , 2nd ++ , 4th +—, 16th to 21st +—, 24th +—, 41st to 51st +—, 62nd to 64th +— The blood was never negative for 15 consecutive days during treatment

*Spleen*—*Vide* Table I

*Result*—The patient relapsed during the 3rd week of observation after treatment. He was given a course of 28 days' treatment with cinchonine sulphate, after which he was observed for 8 weeks without a relapse

Case No 10—J B, aged 22 years

The patient had a history of four previous attacks of malaria

*Treatment*—The patient received 681 pills of Peracrina during a treatment lasting 68 days. Twenty grains of quinine were given on one occasion

*Temperature*—Rigors occurred on the 1st, 3rd and 5th days of treatment with temperatures of 100.5° to 102°F. Another rigor occurred on the 14th day (102°F), and 20 grains of quinine were given. No further fever was recorded until the 28th to 31st days during which fever and rigors were present (101° to 102°F). No subsequent febrile attacks were observed during treatment

The methods used in carrying out this experimental work was similar to that already described in a previous article of this series (Sinton, 1926). Daily blood examinations were made by the thick film method in all cases during the course of treatment. The size of the spleen was examined each week or oftener if considered necessary. The patients were weighed each week, and the greatest care was taken to see that the treatment prescribed was actually taken.

After the cessation of all treatment the blood of each patient was examined weekly for 8 weeks to determine the occurrence of parasitic relapse (Sinton, 1926).

#### MEDICAL HISTORIES OF THE CASES

The patients were young adult British soldiers and were in a well-nourished condition. Nine of the patients had from 3 to 10 previous relapses of benign tertian malaria and one (Case 2) had a history of only one previous attack of malaria.

##### Case No 1—A. R. H., aged 25 years

This patient had a history of numerous previous attacks of malaria, three of which had occurred while under our observation.

*Treatment*—This was carried out as described above and continued until the 113th day, during which time the patient had taken 1,248 pills of Peracrina. Twenty grains of quinine sulphate in solution was given on two occasions on account of severe rigors or the occurrence of very numerous parasites in the peripheral blood.

*Temperature*—The patient had a rigor on the 5th and 7th day of treatment with a temperature of over 103°F on each occasion. He was given 20 grains of quinine in solution on the 8th day, after which his temperature remained normal until the 28th to 30th days on each of which he again had rigors with temperatures of 102° to 103°F. Twenty grains of quinine were given on the 30th day after which his temperature remained normal till the 93rd day, when he had a temperature of 100°F. No further rise of temperature was recorded during treatment which ended on the 113th day.

*Parasites\**—Before treatment ++++, 2nd ++, 5th to 7th +++, 8th +++++, 9th +—, 10th +, 16th +—, 22nd to 28th +—, 29th +++, 30th +++++, 31st ++, 51st to 53rd +—, 55th +—, 65th +—, 67th to 69th +—, 70th and 71st +, 72nd to 74th +—, 78th to 80th +—, 82nd +, 83rd +—, 85th to 89th +—, 91st to 94th +—, 106th to 110th +—. The blood was negative for only 3 days before the end of treatment, and was never negative for more than 11 days consecutively during treatment.

*Spleen*—*Vide* Table I

*Result*—No relapse was recorded.

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\* The numbers refer to the days of treatment, and when a day is not recorded it means that the blood examination on that date revealed no parasites. The symbols ++++ denote 'numerous parasites', +++ a 'fair number of parasites' but less than +++++, ++ means 'scanty parasites', + 'very scanty' parasites, and +— 'extremely scanty' parasites.

9 days) Another series of seven cases was each given a single dose of 30 grains of quinine and amongst them the average apyrexial period was 18.3 days (maximum 48 days, minimum 3 days) These results are very similar to those seen in the Peracrina cases

The fact that febrile attacks may occur during Peracrina treatment is a serious drawback, more especially in an uneducated population, whose demand is for a quick clinical cure and who know that quinine in appropriate doses will produce this

**B Parasites**—Four of the patients with benign tertian malaria, whose medical histories are recorded by Walker (1924), had asexual parasites in their peripheral blood as late as the 86th, 60th, 76th and 38th days of treatment respectively, and gametocytes up to the 56th, 36th, 76th and 35th days In his next two cases the duration of parasites seems to have been 18 and 54 days, while in the last two both sexual and asexual forms were present up to the 15th and 21st days, at which times it was found necessary to resort to quinine treatment on account of the severity of the symptoms

Fletcher (1925) found that 8 pills of Peracrina given daily to adults did not clear the peripheral blood of parasites in 10 to 14 days in the majority of patients

Amongst our patients parasites were recorded as late as the 110th, 106th, 22nd, 69th, 33rd, 79th, 74th, 73rd, 64th and 53rd days of treatment, in spite of a continuous daily dosage of 12 pills of Peracrina from the 11th day onward and of quinine in addition on several occasions when the symptoms were severe In Cases 1 and 2 parasites were present over considerable periods in spite of the administration of 15 pills daily

The presence of parasites in the peripheral blood over such long periods may be due perhaps, as asserted by Walker (1924), to the provocative action of Peracrina but no evidence was found to support this The findings rather

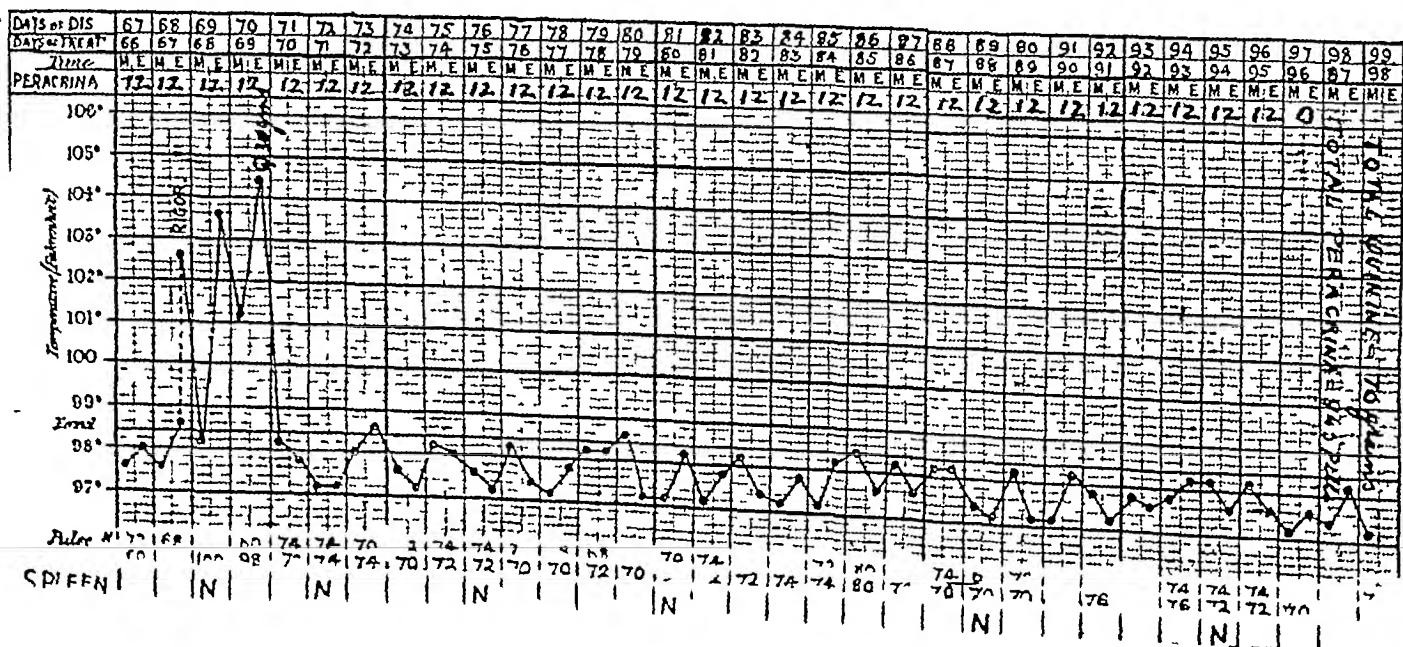
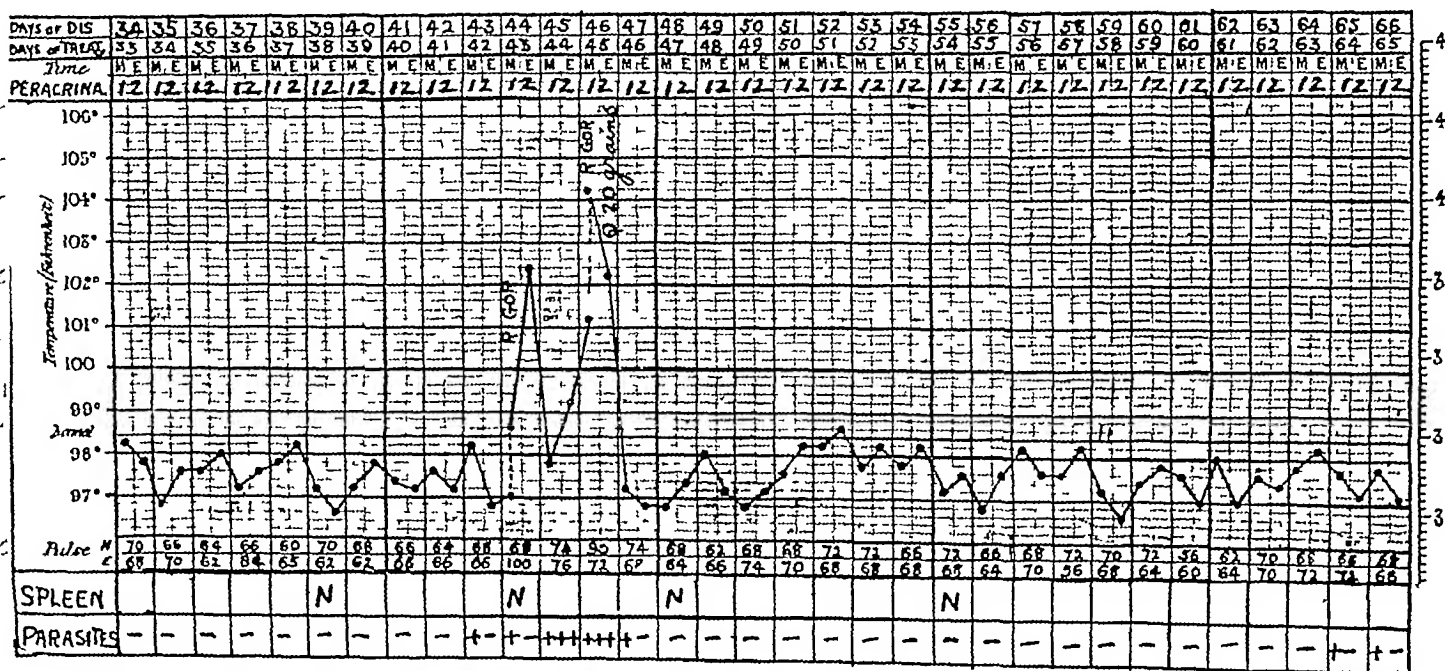
TABLE I

*Results of Splenic Examinations during weeks —*

Case No	Before	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	3F	3F	4F	1F	P	5F	3F	1F	N	N	N	N	N	1F	2F	P	N
2	N	N	N	P	3F	1F	N	N	N	N	N	N	N	N	N	N	N
3	1F	3F	N	1F	3F	P	N	N	N								
4	N	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	
5	N	N	N	N	N	N	N	N									
6	3F	4F	5F	3F	3F	2F	2F	P	P	P	P	P	P	P	P	P	
7	N	N	N	P	N	N	N	N	P	N	N	N	N	N			
8	1F	1F	N	1F	P	P	N	P	P	P	N	N					
9	N	N	N	N	N	N	N	N	N	N	N	N					
10	N	1F	P	N	N	P	N	N	N	N	N						

*Explanation* N = Normal spleen P = Palpable spleen 1F, 2F, 3F, etc = 1, 2, 3 etc., finger-breadths of enlargement of the spleen beyond the costal margin

NAME		J. M. (Case 4)		Age		25		Disease		BENIGN TERTIAN MALARIA		Result		No Relapse																				
DATE OF DIS.	TIME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
DATE OF DIS.	TIME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
DATE OF DIS.	TIME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
DATE OF DIS.	TIME	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
TRACING		0	6	6	6	8	8	8	8	9	9	9	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
TEMPERATURE (Fahrenheit)		106°	105°	104°	103°	102°	101°	100°	99°	98°	97°	96°	95°	94°	93°	92°	91°	90°	89°	88°	87°	86°	85°	84°	83°	82°	81°	80°	79°	78°	77°	76°	75°	74°
PULSE		88	68	74	82	76	68	68	54	62	70	72	64	72	56	58	62	64	64	66	68	66	66	72	100	98	58	68	60	68	72	64	62	60
SPLLEN		N		P								P					P				N	N		N									N	
PARASITES		+++	++	+++	+	-	+	-	-	-	-	++	+	-	-	-	-	-	-	-	+	+	-	+	++	+	+	+++	-	-	-	-	-	-



two relapsed, one although no parasites could be found during 34 consecutive days of treatment. The other four patients were treated from 72 to 112 days (average 87 days) without giving 15 consecutive negative blood tests. The treatment was then stopped in these cases because our supply of the drug became exhausted. Two of these patients relapsed later, including the patient who had been treated for 112 days.

G *Dosage*—The treatment used started with 6 pills daily and worked up to 12 pills daily on the 11th day. This dose is higher than that recommended by the makers, except in attacks of fever. This larger dose was given in consideration of the fact that Walker had given 8 to 12 pills daily to children of 12 to 13 years. Neither of the cases recorded here who received 15 pills daily over long periods relapsed.

H *Complaints by Patients*—A number of the patients complained of nausea, listlessness and loss of appetite, more especially during the early stages of treatment. Walker (1924) records a case in which the treatment had to be stopped on account of severe vomiting.

The patients also objected to the tediousness of having to swallow 12 large pills daily over periods of two or three months, more especially as this treatment did not seem to control their attacks in the same way that quinine did. For this reason the patients expressed a desire to be placed on quinine treatment instead.

The uncertainty of the duration of this treatment had also a depressing effect on the patients.

I *Expense of Treatment*—The present price of Peracrina 303 would preclude the use of this drug as a routine method of treatment, even if the results had been more encouraging.

### CONCLUSIONS

1 The results of the experiment detailed above does not bear out the statement of Walker (1924) that Peracrina 303 is 'a specific remedy against malaria well suited to replace or supplement quinine in many cases'.

2 Even if Peracrina 303 has a beneficial effect in chronic malaria, the cost, the length of the treatment required and its uncertainty make it unsuitable for routine work as compared with quinine.

### THANKS

Our thanks are due to Mr P O R Bally, the representative of the Haco Company in India, through whose kindness we were supplied with free samples of Peracrina to carry out this experiment.

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*Parasites*—1st and 2nd ++, 3rd +—, 33rd +—

*Spleen*—*Vide* Table I

*Result*—No relapse was recorded during observation

Case No 6—G A, aged 22 years

The patient had a history of five previous attacks of malaria

*Treatment*—The patient received 921 pills during the 93 days of treatment. He was given quinine in solution on four occasions during treatment, a total of 90 grains

*Temperature*—Rigors with temperature of 101°F to 104°F occurred on the first three days of treatment, for this reason 20 grains of quinine were given on the 3rd day, after which the temperature remained normal until the 20th day. On the 20th and 21st days rigors again occurred with temperature of 102° to 103°F and 20 grains of quinine were given on the latter day. The temperature rose to 102.2°F on the 22nd day, after which it remained normal till the 42nd day. Rigors were present again on the 42nd, 43rd and 45th days with temperatures of 101.5° to 103.8°F, but no quinine was given. The temperature was then normal till the 57th day. On this day and on the 59th day rigors with fever of over 103°F occurred of such severity that 20 grains of quinine were given on the 59th day. The temperature remained normal till the 75th day. On the 75th, 76th and 77th days rises of temperature were recorded in the evenings, varying between 99.5° and 101.8°F but on the 78th day a rigor with fever of over 102°F occurred and 30 grains of quinine were given. From this time till the end of treatment the temperature was normal

*Parasites*—1st +++++, 2nd +—, 3rd +++, 18th +—, 19th +, 20th ++, 21st +++, 22nd ++, 26th +—, 42nd +—, 43rd ++, 44th and 45th +—, 51st and 52nd +—, 56th to 59th +—, 60th +—, 63rd and 64th +—, 71st +—, 74th +—, 76th +—, 78th +—, 79th +—

*Spleen*—*Vide* Table I

*Result*—The patient relapsed during the 5th week of observation after treatment. He was given a course of 28 days' treatment with cinchonine sulphate, after which he was observed for 8 weeks without a relapse

Case No 7—P H, aged 23 years

The patient had a history of three previous attacks of malaria

*Treatment*—The patient received 885 pills of Peracrina during the 89 days of treatment. He also received 30 grains of quinine in solution on two occasions during treatment

*Temperature*—A rigor with fever of over 101°F occurred on the 4th day. Again on the 16th and 18th days there were rigors with temperatures of 101° to 102°F. Thirty grains of quinine in solution were given on 17th day. Another rise of temperature was present from the 29th to 32nd days due to a congested condition of the lungs and the patient was given 30 grains of quinine at this time. Another rigor occurred on the 80th day possibly due to a small cutaneous abscess

found that a single injection of sodium stovarsol was sufficient to stop a series of febrile paroxysms in benign tertian malaria. It also caused a rapid disappearance of *P. vivax* from the peripheral blood of patients, who had been artificially infected with this fever in the treatment of general paralysis of the insane. Marchoux (1925) gives fuller details of these experiments, in which a single injection of 1 gram of sodium stovarsol was given by the intravenous route to seven patients who had been artificially infected with *P. vivax*, and also to two patients who had acquired this infection naturally in Africa. These injections caused the disappearance of the parasites from the peripheral circulation in 24 hours in all the cases, and are stated to have prevented relapse in six of the patients during an observation period of 2 months. He then tried the drug in Algiers on patients, mostly children, suffering from natural infections with *P. vivax*, and again found the same marked action on the parasites, but he gives no information as to the effects of this treatment in preventing relapses at a later date.

This seemed a very promising line of treatment, so we started, in the spring of 1926, a research into the effects of the intravenous injection of sodium stovarsol in the treatment of benign tertian malaria, following on the experiments already recorded on the oral administration of stovarsol (Sinton, 1926c). While these experiments were in progress, several other workers recorded results obtained by this form of treatment, but the number of patients treated and the details published are mostly few.

Ciuca and Alexa (1926) commenced with a preliminary dose of 0.25 gram of the drug intravenously and continued the treatment by daily injections, without exceeding a daily maximum of 1.5 grams. Unfortunately, no precise details are given of the duration of the treatment or the total amount of the drug administered by this method. They found, however, that the schizonts and gametocytes of *P. vivax* disappeared from the peripheral blood, even after a single minimal dose of 0.25 gram of the drug. They also reported that all the cases receiving less than 1.0 gram relapsed during the first 10 days after treatment ceased. Vialatte (1926), however, records his cases in greater detail. He gave the drug in doses of 1.0–1.25 grams intravenously at intervals of 6 or 8 days for three doses. Five cases of benign tertian malaria were treated in this way, and of these, three were lost sight of immediately after treatment, the fourth relapsed in the interval between the 2nd and 3rd injections and again 13 days after the 3rd dose, while the fifth patient relapsed 17 days after the last injection. These results were so discouraging that Vialatte resumed the oral method of administration of the drug, because he considered that a more prolonged treatment was necessary.

Mazza, Cossio and Aybar Albarracín (1926) report that in 17 patients with a single infection of *P. vivax*, one injection of 1.0 gram of sodium stovarsol was sufficient to clear the peripheral blood of parasites for a month, while in one patient with a double infection, two injections were needed to produce this result. Gravot (1926), writing from Morocco, records the treatment of 13 patients suffering from benign tertian malaria by means of intravenous or intramuscular injections of the same salt. He reports that a single injection will accomplish a radical cure and advocates the intravenous administration of a dose of 0.5–1.0 gram.



*Parasites*—1st to 3rd +++, 4th +—, 6th + —, 8th + +, 9th to 14th +—, 28th +, 29th +++; 30th and 31st ++, 32nd +, 33rd +—, 50th and 51st +—, 53rd +—

*Spleen*—*Vide* Table I

*Result*—No relapse was recorded during observation

#### DISCUSSION ON THE RESULT OF TREATMENT

A *Fever*—It can be seen from the details given in this paper and from the work of Walker (1924) and of Fletcher (1925), that patients, while undergoing treatment with large doses of Peracrina 303, often have acute febrile attacks. These attacks may be so severe that quinine has to be given to control them, indeed, Walker (1925) had to abandon Peracrina treatment entirely and resort to quinine only, in about 20 per cent of his cases, because the 'provocative action' of Peracrina was said to be so severe.

One of Walker's charts recorded a febrile reaction as late as the 48th day of treatment, and, among the patients whose histories are recorded in this paper, febrile attacks were present in some even up to the 78th to 80th days of treatment. As can be seen from the medical histories, recrudescences of fever occurred among our patients even during periods when 12 pills were being taken each day, although this the dose recommended by the makers to reduce fever. In Cases 1 and 2, however, no rise of temperature was observed while 15 pills were being taken daily over periods of 7 and 12 weeks respectively.

In any discussion of the effects of Peracrina treatment in preventing febrile attacks in malarial patients, one must take into consideration the experience of a large number of workers, that many cases of benign tertian malaria, more especially those of a chronic nature, will become afebrile 'if rested in bed and suitably dieted, even in the absence of any specific treatment'.

The temperature charts in our cases resemble in many respects those given by James (1920) for cases of untreated malaria. The earlier observations of Caccini (1902) are also of interest, for he found that 'every spring tertian which is left to itself without the intervention of quinine treatment, after a certain number of more or less regular febrile attacks, will in a certain number of cases present a sudden cessation of attacks. In other cases the attacks become irregular or simply increasingly rare, until they finally disappear'.

Our cases are not quite comparable to such cases for they mostly received some quinine treatment at the times of fever. For comparison 14 cases of chronic benign tertian malaria, under conditions similar to the Peracrina cases, were each given a single dose of 20 grains of quinine in solution at the time of a febrile paroxysm. In all of these, except one, the temperature fell to normal inside two days, while the other case required a further dose of 30 grains of quinine. These cases received no further treatment and were carefully observed until a febrile relapse occurred. It was found that in these cases the average apyrexial period was 19.7 days (maximum 62 days minimum



(1926), containing 15 drms (60 grms) of sugar and 15 grains (1 gm) of sodium bicarbonate, was given by the mouth an hour before each injection and repeated one hour after the injection to protect the liver cells (Sinton and Hughes, 1924). All the patients were confined to bed while the course of treatment was in progress.

The patients in this group were divided into 3 series, which received the treatment recorded below.

1 *Treatment S S 1*—A single dose of 10 gm of sodium stovarsol was given.

2 *Treatment S S 2*—The patients undergoing this treatment were given 10 gm of the drug on the first day, and the same dose was repeated on the third day, a total of 20 grms.

3 *Treatment S S 3*—In this series, 10 gm of the drug was given on the first day, 15 gm on the third day, and a similar dose on the fifth day, a total of 40 grms.

#### CONTROL GROUP

Four series of control treatments were used, and as in previous experiments, the patients were allotted to the different series in strict rotation.

1 *Treatment Q C M*—These patients were treated with the quinine sulphate, citric acid and magnesium sulphate mixture, already described (the 'Mist Q' of Sinton, 1926*b*). This mixture was given so that the patients received 30 grains (2 grms) of the quinine salt daily for the first 2 weeks, and 10 grains (0.6 gm) daily for the next 6 weeks. The total amount of quinine taken by each patient was 1,190 grains (77 grms) \*.

2 *Treatment M C M*—These patients were treated with 'Málarene,' which is a standardised 'cinchona febrifuge' prepared by the Director of Agriculture (Cinchona), Madras †. 'Málarene' was administered in a mixture similar to the quinine mixture mentioned above and it was given to each patient in amounts equivalent to 20 grains (1.3 grms) of total alkaloids daily for 28 days. The total amount of the drug taken by each patient was, therefore, 560 grains (36.5 grms).

3 *Treatment C C M*—The patients in this series were given a mixture similar to that in the Q C M series, except that the quinine was replaced by cinchonine sulphate. The dosage of the mixture was such that each patient received 20 grains (1.3 grms) of cinchonine daily for 28 days. The total amount of cinchonine sulphate taken by each patient was 560 grains (36.5 grms).

\* This treatment is almost the same as the 'standard treatment' of Bass (1922), except that the initial dosage of 30 grains daily was continued for a longer period in our experiments.

† 'Cinchona febrifuge' is a mixture of the total alkaloids in the bark of *Cinchona succirubra* and, therefore, contains quinine, cinchonidine, quinidine, cinchonine, quinidine, and various impurities. The usual commercial cinchona febrifuge varies in its composition, and in the preparation referred to above, an attempt has been made to standardise it.

suggest those seen in chronic cases of benign tertian malaria which are untreated or received treatment only at the times of febrile attacks

The presence of gametocytes in the peripheral blood over such long periods is a much more serious consideration, for it means that patients taking Peracrina treatment may be potential sources of malarial infection in the mosquito and so of the spread of the disease. If this be compared with quinine treatment, our experience and that of many other workers has been that 30 grains of quinine daily in solution will almost invariably free the peripheral blood of sexual forms of *P. vivax* in four days and that 10 grains of quinine daily after this will prevent their reappearance, as long as this amount is being taken daily. The fact that such quinine treatment, so long as it is carried out, will render patients harmless as far as the spread of the disease is concerned is a very important consideration.

C *Spleen*—Walker (1924) records splenic enlargement in many of his cases and the after-histories of his patients show that the enlargement may persist over long periods, in spite of his treatment, and that it may even increase at times. This has also been our experience with Peracrina, while our experience with quinine treatment in a very large series of cases has been that quinine causes a steady decrease in size of the organ, and that the decrease occurs much more rapidly than with Peracrina (Table I). The presence of splenic enlargement is an important factor, especially amongst army patients, who cannot usually be given anything but very light duty in the presence of such enlargement.

D *Weight*—The weights of all the patients were taken each week and in spite of the fact that these patients were receiving extra diet in most cases, it was found at the end of treatment that eight of them had lost on an average  $3\frac{1}{4}$  lb each, while the other two had gained on an average  $2\frac{1}{4}$  lb each.

Walker (1924) attributes the failure of the drug in some of his cases to the fact that most of the patients treated were from famine areas and hence were extremely weakened by hunger and other privations. This, however, cannot be said of the cases recorded here, who were all well-nourished British soldiers. It seems more probable that the good results obtained by Walker were in part, at least, due to the fact that his ill-nourished patients were put to bed and given a generous diet in hospital.

E *Relapses*—As can be seen from Walker's paper the number of relapses amongst the 40 cases of benign tertian malaria whom he traced for some months was about 48 per cent. Amongst our patients the relapse rate, as determined by weekly examinations of the blood by the thick-film method over a period of 8 weeks after the cessation of all treatment, was 40 per cent, in spite of a treatment which lasted on an average 81 days (49 to 112 days) during which time an average of 843 (465 to 1,248) pills of Peracrina was given to each patient.

F *Duration of Treatment*—The makers of this drug recommend that the treatment should continue until no parasites can be found in the peripheral blood for 15 consecutive days. The time required to achieve this result in six of our patients was from 37 to 93 days (average 72 days), and of these

4 *Treatment C D C M*—Twenty-one relapses were recorded among twenty-seven patients treated in this series. The observed relapse percentage was, therefore, 81.5

### *Discussion of Results*

It can be seen from the data given above that amongst 25 patients, treated with intravenous injections of sodium stovarsol in varying amounts up to a total of 4.0 grms in 5 days, twenty-three had parasitic relapses during the eight-week observation period, i.e., an observed relapse rate of 92 per cent. The average relapse rate among the 108 control patients treated with various cinchona alkaloids was only 73.1 per cent\*. All of the eleven patients, treated with one or two injections only, relapsed, while only two of the fourteen who received three injections of sodium stovarsol did not show parasitic relapses during the period of observation.

These results, unfortunately, do not fulfil the hopes which were raised by Marchoux's work, that two or three intravenous injections of this drug might permanently cure infections due to *P. vivax*. It will be seen by consulting the abstract of the work of other observers, given earlier in this paper, that our results more nearly approximate those obtained by Vialatte (1926) than the more optimistic records of some of the other workers.

### THE EFFECTS OF STOVARSOL ON THE DURATION AND REAPPEARANCE OF *P. vivax* IN THE PERIPHERAL BLOOD

The peripheral blood of all the patients of the stovarsol group was examined by the 'thick-film' method, immediately before the first injection was given, and hourly afterwards until parasites could no longer be found. After this time the blood was examined daily for 5 weeks, and weekly afterwards up to the end of 8 or 9 weeks, if parasites were not detected sooner.

*Duration of parasites in the peripheral blood*—The average duration of parasites in the peripheral blood after the first injection of stovarsol in the 25 patients was 19.8 hours (max 30 hours, min 5 hours). All the parasites had disappeared before the time of the second injection. No relationship could be found between the numerical prevalence of parasites in the peripheral blood and the rate of disappearance.

*Time of reappearance of parasites*—In the case which received only one injection, the parasites were again detected after 72 hours. Among the 10 patients who received 2 injections, parasites were absent on an average 20.3 days (max 37 days, min 12 days) after the last injection. Of the 14 patients, who were injected thrice, two did not show parasites again during the observation period.

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\* The cure rate of only 25 per cent obtained in the quinine control series is very far removed from the 90 per cent cure rate claimed for the 'standard treatment of Bass,' in which even a smaller total amount of quinine is given. It is possible, however, that if malignant tertian infections (*vide* Sinton, 1926b), or fresh infections of benign malaria were treated over such long periods that the 90 per cent rate claimed by Bass (1922) might be more nearly reached.

# STUDIES IN MALARIA WITH SPECIAL REFERENCE TO TREATMENT

## Part VII.

### THE INTRAVENOUS INJECTION OF SODIUM STOVARSOL IN THE TREATMENT OF BENIGN TERTIAN MALARIA

BY

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IN a previous paper of this series (Sinton, 1926c) the results of experiments on the treatment of benign tertian malaria by the oral administration of stovarsol were described. The results which were recorded in that paper help to confirm the statements of other workers that this drug, when given by the mouth, will produce the rapid disappearance of *Plasmodium vivax* from the peripheral blood and also a 'clinical cure' of the disease. Unfortunately in the dosage used in our experiments, the claim that this method of administering the drug will produce a 'true cure' (i.e., absence of subsequent relapses) was not borne out. For this reason we have tried the sodium salt of the drug by intravenous injection, in the hope that better results in the prevention of relapse might be obtained.

#### OTHER WORK ON THE INTRAVENOUS INJECTION OF SODIUM STOVARSOL IN BENIGN TERTIAN MALARIA

Marchoux and Cohen (1925) first reported the beneficial effects of the intravenous injection of this drug in the treatment of malaria. These workers

( 287 )

very faintly and was irregular in shape and small in amount. The chromatin retained its staining power much longer.

The impression which we got from a study of our preparations was that the parasite was being dissolved off the surface of the cell, rather than inside the cell as stated by Marchoux (1925).

## THE EFFECTS ON CLINICAL MANIFESTATIONS

### A Clinical Cure

The claim that a 'clinical cure' can rapidly be produced by the intravenous injection of sodium stovarsol, appears to be substantiated by our observations. The intravenous injection of two doses of this drug led to the disappearance of parasites from the peripheral blood and the absence of clinical symptoms for periods varying from 11 to 37 days.

This action of the drug might be of considerable use in the treatment of the acute symptoms of the disease, in patients to whom, for any reason, the administration of the cinchona alkaloids was contra-indicated.

### B Temperature

*Duration of fever*—As in our experiments with the oral use of this drug (Sinton, 1926c), we have used the term 'fever' to denote an oral temperature of 99.5°F or over, and the 'duration of fever' to be the interval which elapsed between the first administration of treatment and the time when the temperature fell below 'fever' level and remained down with no further febrile rise during treatment. The temperatures were taken as a routine, daily at 7 a.m. and 4 p.m., but more frequent observations were made when 'rigors' or other febrile symptoms were present or suspected. The 'duration of fevers' was measured in periods of half a day.

Twenty patients or 80 per cent of the experimental group, showed 'fever' after the commencement of treatment, as compared with fifty-four patients or 50 per cent in the control group. The average 'duration of fever' among the patients treated by the intravenous injection of stovarsol was 0.52 days per case, as compared with 0.29 days in the control group.

*Rigors*—We have used the term 'rigor' to denote a rise of temperature of over 100°F accompanied by a distinct shiver. In the experimental group, nineteen patients or 76 per cent developed rigors within 24 hours of the commencement of treatment, as compared with forty-eight patients or 44.4 per cent among the control group.

Amongst the experimental cases, the maximum temperatures recorded in two cases were 105.1°—106°F, in two cases 104.1°—105°F, in seven cases 103.1°—104°F, in four cases 102.1°—103°F, in three cases 101.1°—102°F, and in one case 100.1°—101°F, as compared with one, four, fourteen, seven, thirteen and nine respectively, in each of these groups in the control cases. If these figures be analysed it will be seen that in 44 per cent of the experimental cases the maximum temperature lay between 103.1°F and 106°F as compared with 17.6 per cent in the control group.

of the drug, which he says suffices to clear the blood of parasites for several weeks

#### DETAILS OF METHODS, ETC, USED IN THE EXPERIMENTS

The routine procedure used during these experiments and the methods of control, observation for relapse, etc., were similar to those already described in previous articles of this series (Sinton, 1926*a*, 1926*b*)

All the patients were young British soldiers of about 20—30 years of age, whose sojourn in India had averaged about 5 years each. The experimental and control groups of patients were housed together and as far as possible they lived the same existence, i.e., did the same amount of work, took the same amount of exercise in the form of games, parades and physical drill, and consumed the same diet. All the patients were suffering from chronic infections with *P. vivax* only, which had been treated with quinine on other occasions. They had previously relapsed on an average four times each. No patient was treated unless *P. vivax* had been found in his blood immediately prior to the commencement of treatment.

As in the previous experiments recorded in this series, the patients were treated and observed in a hill-station (Kasauli), under conditions which precluded any chance of reinfection during the course of the experiments (Sinton, 1926*a*)

#### TREATMENT

The patients were divided into two main groups—an experimental group and a control one. As in previous work the patients were allotted to the different series in strict rotation to avoid any personal bias in the selection of the cases. The average number of previous relapses in the experimental group was 4.4 per patient, as compared with 4.2 in the control group, so there was little difference in this respect between the two groups.

A preliminary purgation with calomel, followed by magnesium sulphate, was given as a routine measure to all patients before the commencement of any form of treatment.

#### EXPERIMENTAL GROUP

The patients in this group were treated with intravenous injections of sodium stovarsol in the varying doses detailed below. Each dose of the drug (1.0—1.5 grms.) was dissolved in 10 ccs. of sterile distilled water before being injected into the veins in front of the elbow. No other drug was used in the treatment.

The risk of arsenic having a poisonous action, on some of the different organs and tissues following the injection, was not overlooked. With a view to preventing such dangers, the free elimination of arsenic from the bowel was ensured by the administration of 60 grains (4 grms.) of magnesium sulphate daily during treatment. In addition the solution recommended by Harrison

10 a m and in every case the temperature had fallen to normal before 7 a m on the following morning. In only one case did the temperature again rise above 99.5°F after this time. This was in a very chronic infection with *P. vivax* in which the temperature rose to 100.6°F on the day following the first injection, but was normal on the next day with no subsequent febrile rise.

A careful study was made of the hour and day on which fever occurred among the patients before treatment was commenced. No relationship, however, could be traced between the febrile reaction which followed the intravenous injection, and any febrile reaction produced by the parasites on either of the two days preceding the commencement of treatment. One patient had a temperature of 103°F due to a malarial paroxysm on the day previous to his injection and his fever had fallen to normal by 10 a m the next morning when the injection was being given, but it rose again to 103.2°F less than 3 hours later.

One patient, who was probably at the commencement of a malarial paroxysm, had a temperature 99°F when his first injection was given. His temperature rose to 102.6°F in 80 minutes, was 104°F after 2 hours and 105.8°F after 4 hours, but had fallen to normal again in less than 9 hours. A second injection given to this patient two days later, failed to provoke any febrile reaction. A similar case, in which the oral administration of stovarsol at the commencement of a malarial paroxysm provoked an excessive reaction, was noted in a previous paper (Sinton, 1926c).

No definite relationship could be made out between the numerical prevalence of malarial parasites in the peripheral blood before the injections, and the degree of febrile reaction provoked by the treatment. The degree of febrile reaction in malaria, other things being equal, is probably proportional to the amount of malarial protein liberated into the general circulation at any one time. The evidence available seems to indicate that stovarsol has a destructive action on all stages of development of *P. vivax*, but more especially on the larger forms. The amount of malarial protein liberated by the action of the drug will, therefore, depend on the actual size of the parasites destroyed as well as their numerical prevalence, so a small number of fully developed parasites might produce a larger amount of foreign protein than a greater number of very small parasites. It is, of course, impossible to estimate the amount of malarial protein liberated, but in our cases the degree of febrile reaction seemed to be greater when large parasites were present before injection, than when there were present only small forms in equal number. The febrile reaction was usually greater also when there was a double infection than when a single one was present. This may explain the failure to discover any definite relationship between the numerical prevalence of parasites and the degree of febrile reaction.

These results confirm the previous findings that stovarsol has a provocative action on temperature, and indicate that the drug should be administered with caution when it is suspected that a malarial paroxysm is imminent. The provocative effect is probably due to the large amount of 'toxin' liberated by the great destruction of all stages of the parasite. This point has been discussed in another paper (Sinton, 1926c).

4 *Treatment C D C M*—These patients were treated exactly like the last series except that cinchonidine sulphate was used instead of cinchonine sulphate. The total amount of the drug given was 560 grains (36.5 grms) to each patient during a period of 28 days.

#### THE EFFECTS OF TREATMENT IN PREVENTING RELAPSE

As in our previous experiments (Sinton, 1926a), the criterion of 'relapse' was the finding of parasites in the peripheral blood, irrespective of the occurrence or absence of associated clinical symptoms. The 'thick-film' method was used to examine the blood in all cases. In the experimental group, daily blood examinations were made up to the 35th day and weekly ones subsequent to this time. The examinations were continued up to the end of 8 weeks after the cessation of all treatment or until 'relapse' was detected, whichever happened first. In the control group, weekly blood examinations only were made, up to the end of the 8th week of observation after treatment. Any patient who did not 'relapse' during this observation period of 8 weeks was considered as 'cured'. This point was discussed in full in a previous paper (Sinton, 1926a). All the relapses recorded here were due to *P. vivax*.

#### Results in the Experimental Group

1 *Treatment S S 1*—The only patient included in this group developed jaundice after his first injection of stovarsol, so it was thought inadvisable to give any further injections. He relapsed on the 3rd day after the cessation of treatment.

2 *Treatment S S 2*—This series contained 10 patients, all of whom relapsed during the period of observation. The relapses were first detected on the following days of observation after the completion of treatment—one on each of the 12th, 13th, 15th, 16th, 22nd, 31st, 35th and 37th days and two on the 11th day. The total relapse rate was therefore 100 per cent.

3 *Treatment S S 3*—Fourteen patients were treated in this series, and of them 12 relapsed during the period of observation. These relapses were first detected on the following days of observation after the end of treatment—one on each of the 10th, 14th, 15th, 24th, 42nd and 59th days and three on each of the 12th and 13th days. The remaining two patients, who did not relapse, completed eight and nine weeks of observation, respectively. The observed relapse rate was, therefore, 86.7 per cent.

#### Results in the Control Group

1 *Treatment Q C M*—Twenty-one out of the twenty-eight patients undergoing this treatment relapsed during observation, making an observed relapse rate of 75 per cent.

2 *Treatment M C M*—There were 16 relapses recorded amongst the 26 patients in this series—an observed relapse rate of 61.5 per cent.

3 *Treatment C C M*—Among the twenty-seven patients in this series twenty relapsed, giving a relapse rate of 74.1 per cent.



administration It is advised that the amount of alkali should be increased in patients receiving stovarsol treatment over longer periods

The indirect toxic action of the drug has been discussed in the section dealing with its provocative effects

### CONCLUSIONS

From the treatment of 25 cases of chronic benign tertian malaria, the results obtained under the conditions of our experiment, lead us to arrive at the following conclusions —

1 That the intravenous injection of sodium stovarsol in varying doses up to a total of 4 grms during 5 days have only produced a 'true cure' in a very small percentage of cases and has not confirmed the encouraging results claimed by other workers in this respect

2 That the intravenous injection of 1 grm of sodium stovarsol will cause the disappearance of *P vivax* from the peripheral blood and a 'clinical cure' in the majority of cases Such treatment may, therefore, be of value in acute infections where other drugs are contra-indicated

3 That the drug does not seem to have as rapid an effect in reducing splenic enlargement as have the cinchona alkaloids

4 That the primary injection of stovarsol may have 'provocative action,' evidenced by the production of febrile symptoms in many cases

5 That precautions should be taken to prevent the toxic symptoms which sometimes follow the administration of this drug

My thanks are due to the Director of Medical Services in India for the facilities which he has placed at my disposal for carrying out this work I am unable to express adequately how deeply I am indebted to Major W Bird, R A M C, for the great help which he has given me in conducting these trials

NOTE—Since this article was sent for publication a paper by Guérin, Borel and Adviser (1927) (*Bull Soc Path Exot*, XX, 4, pp 331—337) has come to hand, on the results of the treatment of malaria with intravenous injections of stovarsol The drug was given in a single dose of 1 grm to each case Ten cases of infection with *P vivax*, five with *P falciparum*, three with *P malariae* and one with both *P vivax* and *P falciparum* were treated These workers found that stovarsol did not affect either *P falciparum* or *P malariae*

They found in the benign tertian cases that (a) stovarsol acts on cases due to *P vivax*, (b) all their cases relapsed inside 2 months after the end of treatment, (c) the injection was followed inside 18 hours by a rise of temperature in the majority of cases and (d) even, if the drug does not produce a permanent cure, it has a marvellous and rapid effect in producing a clinical one

Their description of the action of the drug on *P vivax* in the peripheral blood is very similar in most points to that given in this article

They also produce evidence in favour of a 'provocative' action of the drug and found that no aggravation of febrile symptoms occurred after its injection into healthy persons, into persons sick with maladies other than malaria, nor in malaria other than the benign tertian form

of 8 or 9 weeks, while among the other 12 the average number of days during which parasites could not be found was 23.9 (max 59, min 10)

The rapid disappearance of *P. vivax* from the peripheral blood after one injection of stovarsol confirms the findings of Marchoux (1925) and of other workers, but as shown by our results the disappearance is usually only a temporary one and is not the result of the destruction of all the parasites in the body, for these reappear at a later date in the majority of cases

#### THE EFFECTS OF STOVARSOL ON *Plasmodium Vivax*

Marchoux (1926) followed the action of the drug on *P. vivax* in blood slides taken hourly. He says that the action is not rapid but is progressive. The first parasites to disappear were the pigmented forms, which usually could not be found after 6 or 8 hours. The younger forms were more resistant. Ciuca and Alexa (1926) failed to find any difference between the sensitiveness of the small forms as compared with pigmented forms.

Marchoux (1925) states that the process of destruction is not a fragmentation as in the case of quinine. There is first produced a condensation of the protoplasm and of the chromatin. Later, the protoplasm loses the power of retaining stains and melts away. The nucleus at this period is still dense and well coloured and apparently more voluminous than in the normal parasite. It is covered with a thin shadow of the protoplasm. 'It ends by dissolving entirely inside the red blood cell without affecting the latter, for one sometimes sees red cells with Schuffner's stippling but devoid of parasites.'

We followed the course of the destruction of the parasites by hourly thin films in a number of cases. Our experiments bear out Marchoux's contention that this drug has a marked action on the larger pigmented parasites in the peripheral blood. This was especially seen in one case where gametocytes, mostly female, were present in every other field of the thin film before injection and could only be found with very great difficulty at the fourth hour after injection, although ring-forms of the parasite were still present.

The effect on the smaller forms was very curious. The first effect noted, about an hour after injection, was an increase in size of the vacuole, a thinning of the protoplasm and a deeper staining chromatin dot. At the 4th-6th hour the protoplasm of the ring-forms had become very thinned and in many cases was broken up into fine connected strands, sometimes with a ragged, cobweb-like appearance. The vacuole, if present, was very large and the chromatin seemed to be more deeply stained than normal. Another curious finding at this period was a large number of accolé forms, and many times double and treble infections of single cells were observed, although this had not been found before injection. Many of these forms and of the surviving larger forms seemed to be distinctly extracellular in position.

The young unpigmented forms could be found for several hours after the larger forms were no longer detectable, but they were much diminished in number. The action of the drug seemed to prevent the normal increase in size of these young forms, or else destroyed them when they got to a certain stage, so that only small forms could be found. At this period the protoplasm stained



Several of the patients in the experimental group had very marked rigors, which appeared to be more severe than those usually seen in patients suffering from chronic benign tertian malaria in our experience. Among the experimental group one other patient, and amongst the control group ten other patients, showed temperatures of 100°F or less at some time during treatment but developed no distinct rigor.

### C. Splenic Enlargement

Tardres (1926), from the treatment of some cases of benign tertian malaria with stovarsol, concluded that this drug had not the same power in reducing splenic enlargement as had quinine. Ciuca and Alexa (1926) found that they never got complete reduction of the splenic enlargement with stovarsol.

Among our cases the spleen rate was 40 per cent in the stovarsol group before treatment, as compared with 37 per cent in the control group treated with the cinchona alkaloids. At the end of one week from the commencement of treatment, the rates were 20 per cent and 9.2 per cent respectively. The changes noted, in the sizes of the spleen before treatment, as compared with the sizes after one week from the commencement of treatment, were that in the stovarsol group 8 per cent had increased in size, 32 per cent had diminished and 60 per cent had shown no change, while in the control group the percentages were 0.9, 34.2 and 64.9 respectively.

The average enlargement, in finger-breadths beyond the costal margin, of all spleens in the stovarsol group was 0.56 before treatment and 0.2 after one week, as compared with 0.47 and 0.046 in the control group.

These figures go to show that stovarsol has not the same power in reducing splenic enlargement as have the cinchona alkaloids.

### THE PROVOCATIVE ACTION OF STOVARSOL

The opinion which we formed from the oral administration of stovarsol in benign tertian malaria was that 'this drug may possibly have an effect in provoking a febrile reaction' (Sinton, 1926c), and this opinion appears to be confirmed by the results which we have obtained with the intravenous injection of the sodium salt of this drug.

The results recorded while discussing temperature, show that amongst our patients the intravenous injections of stovarsol not only caused a longer average 'duration of fever,' a higher percentage of 'rigors' and a higher degree of temperature than in the control group, but also a larger proportion of cases showed 'fever' after the commencement of treatment.

A feature, which was very noticeable after injection, was the marked and sudden character of both the rise and the fall of the temperature. In one case the temperature rose from normal to 105°F inside 20 minutes from the time of the first injection. The average time which elapsed between the injection and the commencement of a rigor was about 2 hours and the maximum temperature was reached on an average 2 hours later\*. The injections were all made about

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\* It is interesting to note that it is during these first four hours after injection that the greatest destruction of parasites occurs (*vide* The effects of stovarsol on *P. vivax*).

Shipley In the present paper the nomenclature of Nuttall and Shipley is followed, i.e., the anterior portion is termed the *buccal cavity*, and the posterior portion or 'pumping organ,' the *pharynx*

The structure of this part of the food channel is highly complex, and can best be understood by a reference to Plate XXIX, figs 1 and 2

In potash specimens the buccal cavity (BC) is seen to be formed by two plates of chitin, a dorsal and a ventral, which are probably closely approximated in the resting position of the insect. The dorsal plate is thickened anteriorly and posteriorly, whilst the intervening portion is membranous. The anterior thickening (P1) occupies the centre of the roof only, the lateral portions of the latter being membranous. It resembles a trowel in shape, and was called by Nuttall and Shipley the 'hard palate,' and by Thompson the 'anterior hard palate'. It ends posteriorly in a series of transverse folds of chitin, the appearance of which in longitudinal section suggested to Annet, Dutton, and Elliott the term 'taste-papillæ'. From the centre of the anterior hard palate a group of about 4 stout spines projects downwards into the buccal cavity, the 'palatal papillæ' (PP). From 6 to 10 similar spines, the 'dorsal papillæ' (DP) project from the membranous portion of the dorsal plate. These are situated on either side of the anterior hard palate, with the exception of the last pair, which lie posterior to it. Behind the membranous portion the dorsal plate again becomes abruptly thickened, forming the 'posterior hard palate' of Thompson (P2). In the centre of this there is a more darkly chitinated portion, the 'pigmented area' (PIA). The number of the dorsal papillæ and the shape of the pigmented area vary in the different subgenera.

The ventral plate of the buccal cavity of the anophelines is composed throughout of moderately thick chitin, and is carried up laterally to unite with the roof. Posteriorly there projects from its under surface a flange, the 'ventral flange' (VF), which in some species is divided down the centre into two portions. This flange serves for the attachment of the muscles of the salivary pump (SP). A short distance from the point where the buccal cavity joins the pharynx a row of 4 papillæ, the 'ventral papillæ' (VP), projects into the cavity from the ventral plate. At the bucco-pharyngeal junction the lumen of the food channel expands considerably (BPJ), and is bounded laterally by two large chitinous wings, the 'lateral flanges' (LF), arising from the ventral plate. There is a considerable angle at this point, which is more acute in the male than in the female. Dimmock considered that this formed a valve, which he thought prevented regurgitation of fluids into the mouth during the pumping process. The chitinous wall here becomes membranous, and forms a hinge, which allows a certain amount of movement. On the dorsal wall of the bucco-pharyngeal membrane there are usually seen a number of small ridges, the 'bucco-pharyngeal ridges' (BPR), each of which bears from 4 to 6 very minute spines.

In the case of all the Anophelines which we have examined, with the exception of those belonging to the subgenera *Bironella* and *Anopheles*, there is in the female a bucco-pharyngeal armature consisting of a row or rows of stout, chitinous processes or 'teeth' (T), projecting from the thickened posterior end of the ventral plate of the buccal cavity into the lumen of the food channel. These

Yorke and Macfie (1924) have discussed the mechanism of radical cure in malarial fevers and give as their opinion that 'quinine given to a patient whose blood contains numerous malaria parasites invariably destroys directly, or more probably indirectly, large numbers, but not all, of the parasites, thus setting free a considerable quantity of soluble antigen. The antigen provokes by stimulation of the host's tissues the formation of immune-body. The immune-body, if present in sufficient amount, destroys the remaining parasites, thus resulting in sterilisation of the infection and cure of the patient'. If, for any reason, the patient is unable to produce sufficient immune-body he develops a chronic relapsing infection.

This hypothesis is of interest in relation to stovarsol, which by its action on all stages of the parasite should set free a larger amount of 'soluble antigen' than does quinine, which is believed to act mainly on the smaller forms of the parasite. The idea that stovarsol probably sets free a large amount of 'malaria toxin' or 'soluble antigen' is supported by the provocative effects produced by this drug.

If the idea of Yorke and Macfie is correct, we would expect that, in fresh cases of malaria, stovarsol might have a greater curative power than quinine on account of the larger production of antigen. The work of Marchoux (1925) with experimentally infected patients supports the idea that the drug will cure a large number of fresh cases, but this requires confirmation. The poor results obtained in our experiments may perhaps be explained by the inability of these chronic cases to produce immune-body, in spite of a larger production of antigen. Vialatte (1926), however, thinks that the future of stovarsol, as compared with quinine, may lie rather in the treatment of chronic infections than of acute ones.

#### TOXIC EFFECTS OF STOVAR SOL

As with the other arsenical preparations a number of toxic effects of varying degree have been recorded following the administration of stovarsol. Vialatte (1926) notes that different workers have reported colic, diarrhoea, erythema, urticaria, ocular troubles, tachycardia, vertigo and collapse following the use of this drug. He records cases of glycosuria and erythema among his patients. Ciuca and Alexa (1926) found that 9 out of 27 patients treated by them with stovarsol for malaria developed acute nephritic symptoms, which disappeared when the drug was discontinued. For this reason they recommend a daily examination of the urine and that the drug should be stopped on the first indication of renal trouble.

Amongst our patients the only one who showed any signs of toxæmia was the case in Treatment S S 1 who developed jaundice. Although the urine of all our cases was examined before each injection, on no occasion was albuminuria found. The absence of toxic effects in our patients may have been due to the protective action of the glucose solution on the liver (Sinton and Hughes, 1924) and of the alkali on the kidneys (Sinton and Lal, 1924), as well as to the magnesium sulphate purgation.

It, therefore, seems advisable that this prophylactic treatment should be given to all patients treated with stovarsol irrespective of the method of

*Lateral Flanges* vary in size

*Bucco-pharyngeal Ridges* form a single row, and are comparatively poorly developed

A Group *Anopheles* (Plate XXX, figs 5 and 6)

The following species were examined —

*A. atkensi*

*A. culiciformis*

*A. bifurcatus*

*A. plumbeus* var *banianensis*

*A. maculipennis*

*A. lindesayi*

*A. gigas* var *simlensis*

*A. hyrcanus* var *negerrimus*

*A. umbrosus*

*A. barbirostris*

All these species conform to the description given above

B Group *Christya*

No specimens available

C Group *Arribalzaga* (Plate XXX, figs 7 and 8)

Species examined —

*A. punctumacula* (one specimen only)

The whole *Buccal Cavity* and *Pharynx* were on a much larger scale than in Group *Anopheles*. The *Pigmented Area* was wider and more oval in shape, and the *Bucco-pharyngeal Ridges* more poorly developed. The *Lateral Flanges* were very large. In other respects this Group resembles Group *Anopheles* very closely.

#### IV SUBGENUS MYZOMYIA (Plates XXI and XXXII, figs 9 to 22)

The morphology of the buccal cavity and bucco-pharyngeal junction in this subgenus is much more complicated than in any of the other subgenera. The Groups all conform to a similar general plan except in the case of *Neomyzomyia*, which differs from the others so markedly that were the classification of *Anopheles* lines to depend on this feature alone it would apparently form a separate subgenus.

*General Characters* of the Subgenus —

*Bucco-pharyngeal Armature* very complex. As regards the first 4 Groups the appearance of 'rods and cones' noted by Annett, Dutton and Elliott in the case of *A. costalis* perhaps gives the most apt description. The teeth are arranged in two rows, those in the anterior and dorsal row, the 'cones,' having a bulbous base surrounded by a series of short spines (Plate XXXI, figs 11, 15 and 23). From the centre of these spines, as from the middle of a rosette, arises a long tooth curving backwards and upwards. Seen from above the end of the tooth looks blunt and often bifid, but in specimens where a tooth has been separated it is seen to be long and pointed. When separate teeth are viewed laterally, there is seen to be a projection behind the bases of the teeth, which carries a tuft of

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shorter than in the case in the previous group, and their bases are more rounded. They carry two spines, one behind the other (Plate XXXI, figs 16 and 17)

In other details this group conforms to type

*Species examined* —

*A stephensi*

*A fuliginosus*

*A philippinensis*

*A pallidus*

*A maculatus*

*A willmori*

*A theobaldi*

*A jamesi*

*A ramsayi*

*A maculipalpis* var *indiensis*

*A karwari*

C Group *Cellia* (Plate XXXII, figs 19 and 20)

*Species examined* —

*A pulcherrimus*

*A pharoensis*

*A pulcherrimus* resembles the *Neocellia* Group very closely as regards the buccal cavity and bucco-pharyngeal junction. Only one specimen of *A pharoensis* was examined, and this appeared to resemble rather the *Pseudomyzomyia* Group, but a more detailed study of other specimens is necessary.

D Group *Pseudomyzomyia* (Plate XXXII, figs 21 to 23)

*Special Features* —

*Bucco-pharyngeal Apparatus* The 'cones' have long narrow bases anterior to the bulb, and the general alignment of the base from which the teeth arise shows a more pronounced curve than in any other Group examined (Plate XXXII, fig 22)

The *Pigmented Area* (Plate XXXII fig 21) differs in shape from that of the other Groups of this subgenus.

The *Lateral Flanges* are very large.

*Species examined* —

*A subpictus*

*A vagus*

*A ludlowi*

*A ludlowi* var *sundaica*

\**A christyi*

A specimen from Celebes believed to be *A parangensis* was also examined, and appeared to conform to the characteristics of this group.

E Group *Neomyzomyia* (Plate XXXIII, figs 24 and 25)

As mentioned above, the members of this Group differ very markedly from the other Groups placed in this subgenus. They appear to be more closely allied to the subgenus *Nyssorhynchus* than to *Myzomyia*.

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\* This species was provisionally placed by Christophers (1924) in the Group *Neomyzomyia*, but the characters of the buccal cavity in the specimen examined by us clearly place it in the *Pseudomyzomyia* Group.

# THE RELATION OF THE MORPHOLOGY OF THE BUCCAL CAVITY TO THE CLASSIFICATION OF ANOPHELINE MOSQUITOES

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ADLER and THEODOR (1926) drew attention to the peculiar morphology of the posterior portion of the buccal cavity in the genus *Phlebotomus*. Since that time one of us (Sinton, 1927) has confirmed the great diagnostic value of this feature in differentiating the various members of the 'recumbent-haired' group of this genus. It seemed possible that similar features of diagnostic value might also be found to exist in mosquitoes.

## TECHNIQUE EMPLOYED

The specimens were macerated in potash, stained with carbol fuchsin and after dehydration dissected in thin Canada balsam, so as to isolate the buccal cavity and pharynx from the rest of the head. The best preparations were found to be those in which the dorsum of the clypeus was removed. Complete specimens of the pharynx with the buccal cavity attached and of their different component parts were mounted and examined.

## GENERAL DESCRIPTION OF THE BUCCAL CAVITY

The part of the alimentary canal of the mosquito which traverses the head is divided into two portions. The anterior of these, which is directed upwards and backwards from the base of the proboscis, was called by Dimmock (1881) and Thompson (1905) the 'pharynx,' by Annett, Dutton and Elliott (1901) the 'ascending portion of the pharynx,' and by Nuttall and Shipley (1903) the 'buccal cavity'. The posterior horizontal portion, or 'pumping organ' was termed the 'oesophagus' by Dimmock and Thompson, the 'horizontal portion of the pharynx' by Annett, Dutton and Elliott, and the 'pharynx' by Nuttall and

*Species examined —**A. albimanus**A. argyritarsis*B Group *Kerteszia* (*Dendropædium*)

No specimens of this Group were available for examination

## DISCUSSION

The classification of the *Anophelinae* is now recognised to be based on the characters of the male genitalia. The various steps by which the classification used in the last section have been arrived at are discussed by Christophers (1924). His division of the *Anophelini* into the five subgenera—*Bironella*, *Chagasia*, *Anopheles*, *Myzomyia* and *Nyssorhynchus*—seems to be generally accepted.

As may be seen from the description given above, such a classification is largely confirmed by a study of the morphology of the buccal cavity in the female. The subgenus *Bironella* appears to be very closely related to *Anopheles*, as was noted by Edwards (1924). These two subgenera are quite distinct from the others in that they possess no bucco-pharyngeal armature.

The subgenus *Myzomyia* has on general characters been divided into five groups, namely, *Myzomyia*, *Pseudomyzomyia*, *Neocellia*, *Cellia* and *Neomyzomyia*. As regards the morphology of the buccal cavity the first three, though alike in general characters, show certain differences which will require further careful study. With regard to the *Cellia* Group, the only species of which a number of specimens was dissected (i.e., *A. pulcherrimus*) showed very little if any difference from the *Neocellia* Group. The members of Group *Neomyzomyia*, however, show very marked differences from any other Groups in this subgenus, and appear to be nearer to the subgenus *Nyssorhynchus* than to *Myzomyia*.

The character of the bucco-pharyngeal armature in the subgenus *Nyssorhynchus* marks it off very clearly from any of the other subgenera examined.

The specimens examined have been mainly Indian, and it will be interesting to see whether the examination of large series of species from other countries will confirm these findings.

It seems possible that the study of the morphology of the buccal cavity may turn out to be of assistance in differentiating species as well as subgenera and groups, and further work on these lines is in progress.

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| SINTON, J A (1927)                               | <i>Ind Jour Med Res</i> , XV, 1, pp 29—32  |
| THOMPSON, M T (1905)                             | <i>Proc Boston Soc Nat Hist</i> , XXXII, 6, pp 145—200   |

teeth are so arranged that they form a kind of sieve between the buccal cavity and the pharynx, into the commencement of which the former is invaginated at this point. These processes were described by Annett, Dutton and Elliott in the case of *A (Myzomyia) costalis*. Nuttall, Shipley and Thompson noted that they were not present in the case of *A (Anopheles) maculipennis* and *A (Anopheles) punctipennis* respectively.

This armature is absent in the males of all the species of Anophelines which we have examined. As regards Culicines, small 'teeth' are present in *Culex*, but are absent in the other genera so far examined. Further investigations on this point are in progress.

The bucco-pharyngeal armature is a very complex structure, and its exact function is doubtful. Annett, Dutton and Elliott considered that it assisted in the valvular action of this part of the food channel, but they also pointed out that such specialisation in structure would lead one to suppose that it possesses also a sensory function.

#### THE MORPHOLOGY OF THE BUCCAL CAVITY IN THE DIFFERENT SUBGENERA OF ANOPHELINES

We have examined examples of 52 species of Anophelines, representing all the subgenera except *Chagasia*. The results of this investigation are given below, the species being arranged according to the classification given by Christophers (1924).

##### I SUBGENUS BIRONELLA (Plate XXX, figs 3 and 4)

Species examined — *A bironelli* (one specimen only)

*Characters* —

*Bucco-pharyngeal Armature* absent

*Dorsal Papillæ* 12

*Ventral Papillæ* arranged in a group of 4, which seems to be placed on a slightly raised area

*Pigmented Area* elongated, with a circular posterior portion cut off by a narrow waist from the main part

*Posterior Hard Palate* shaped like an inverted egg-cup

*Lateral Flanges* poorly developed

No *Bucco-pharyngeal Ridges* could be made out

##### II SUBGENUS CHAGASIA

No specimens of this subgenus were available for examination

##### III SUBGENUS ANOPHELES (Plate XXX, figs 5 to 8)

*Characters* —

*Bucco-pharyngeal Armature* absent

*Dorsal Papillæ* 8 to 10 (Plate XXX, figs 6 and 8)

*Ventral Papillæ* the usual four

*Pigmented Area* a band extending down the middle of the posterior hard palate

*Posterior Hard Palate* shaped like a truncated cone

EXPLANATION OF PLATE XXIX

- Fig 1 Dorsal view of Buccal Cavity and commencement of Pharynx of an Anopheline Mosquito (Subgenus *Myzomyia*, Group *Pseudomyzomyia*)
- „ 2 Optical median section of Buccal Cavity and commencement of Pharynx of an Anopheline Mosquito (Subgenus *Myzomyia*, Group *Pseudomyzomyia*)

hairs or spines (Plate XXXII, fig 23) The 'cones' are from about 12 to 24 in number The 'rods' (Plate XXXII, figs 12, 16 and 17) are much narrower teeth seen below and behind the intervals between the 'cones' Like the latter they are curved and have pointed ends The bases, which are bulbous, are partly hidden by the bases of the 'cones' In some cases the rods have two spikes, one behind the other (Plate XXXI, fig 17)

The *Dorsal Papillæ* in all the Groups are 6 in number, the posterior pair being usually widely separated from the others

The *Ventral Papillæ* are 4 in number

The *Pigmented Area* varies in shape in the different Groups (*vide* Plate XXXI, figs 10 and 13 and Plate XXXII, figs 20 and 21)

The *Posterior Hard Palate* is more or less rectangular in outline, but in some cases it has a slight transverse constriction about the middle (Plate XXXI, fig 13)

The *Bucco-pharyngeal Ridges* are well-developed, and are seen as rows of wave-like lines behind the teeth From each of these projects a row of small and very slender spines, which from above look short (Plate XXXI, figs 9 and 14 and Plate XXXII figs 19 and 22), but which in dissected specimens are found to be of considerable length (Plate XXXI, fig 18)

The *Lateral Flanges* are in most cases well-developed

A Group *Myzomyia* (Plate XXXI, figs 9 to 12)

*Special features* —

*Bucco-pharyngeal Armature* The 'cones' have a short thick base, which ends in a circle of coarse serrations, from the centre of which arises a long, stout curved tooth The 'rods' are also long and thin On the inner sides of the chitination of the *Lateral Flanges* arise several stout teeth (Plate XXXI, fig 9)

The other structures conform to the general description of the subgenus

*Species examined* —

*A. rhodesiensis*

*A. culicifacies*

*A. histon*

*A. minimus*

*A. aconitus*

*A. jeyporiensis*

*A. turkhudi*

*A. multicolor*

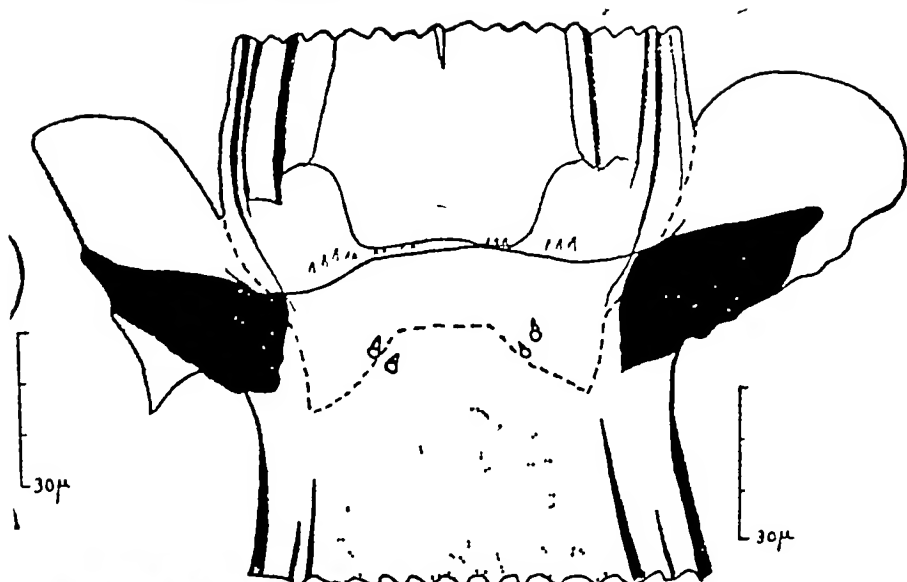
*A. superpictus*

All the above species appear to belong to this Group, except the last three, which will require further investigation

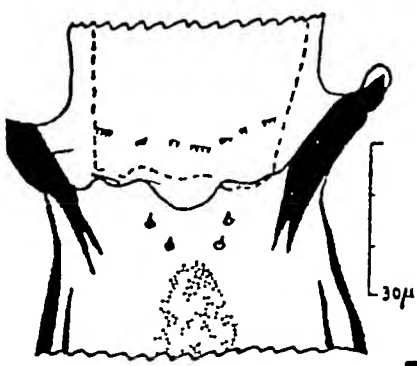
B Group *Neocellia* (Plates XXXI and XXXII, figs 13 to 18)

*Special features.*—

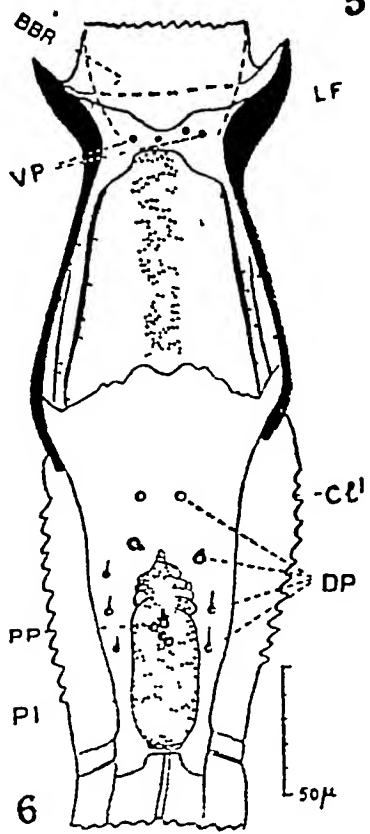
*Bucco-pharyngeal Armature* The 'cones' have a much more rounded base than those in the previous group, and the serrations around their bases are finer (Plate XXXI, fig 15) The teeth, which arise from the centre of the serrations are long and curved, with sharp points and frayed edges Seen from above their turned-up ends appear bifid or trifid (Plate XXXI, fig 13) The 'rods' are



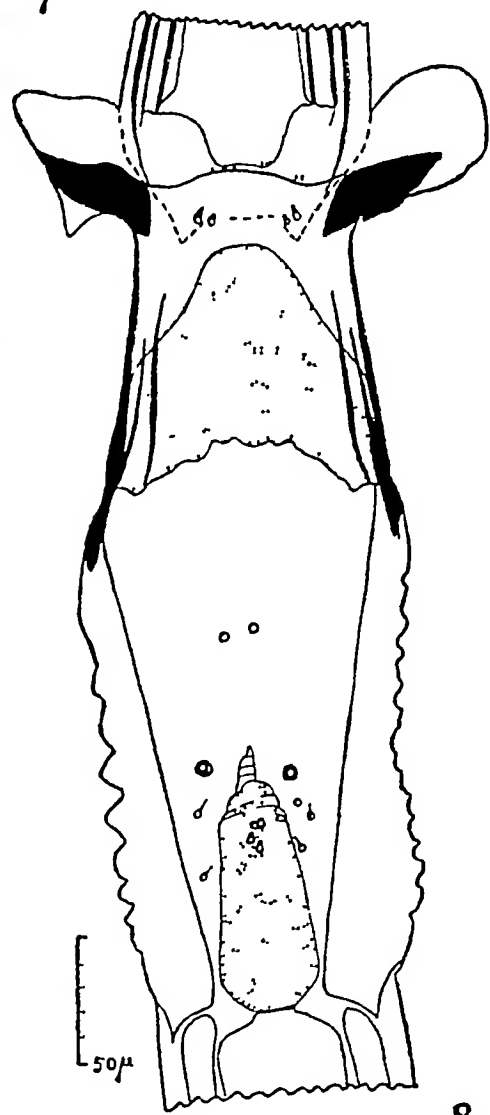
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*Special Features —*

The *Bucco-pharyngeal Armature* has not the 'rod and cone' arrangement of the subgenus *Myzomyia*, but consists of a row of about 10 large pectenate teeth arising from stout bases, separated from one another by distinct intervals. The bases of the teeth carry small spines laterally.

The *Posterior Hard Palate* is usually more highly chitinised than in the other *Myzomyia* Groups, especially in the case of the Australasian members of the Group. In some specimens traces of the 'cobblestone pavement' seen in the subgenus *Nyssorhynchus* can be made out.

The *Bucco-pharyngeal Ridges* are placed in rows posteriorly to the teeth, but are arranged in two groups, one on either side. In the Australasian members of the Group these ridges are developed into short tongue-like projections covered with spines.

*Species examined —**A. aureosquamiger**A. kingi**A. watsoni**A. leucosphyrus**A. tessellatus**A. kochi**A. punctulatus**A. annulipes**A. mastersi* (? = *annulipes*)*A. amictus*V SUBGENUS *NYSSORHYNCHUS* (Plate XXXIII, figs 26 to 28)A Group *Nyssorhynchus**Characters —*

*Bucco-pharyngeal Armature* This consists of two rows of teeth arising from a stout semilunar chitinous plate. The first row is composed of 20 or more small stout sharply pointed teeth with broad bases, disposed in groups of two or three. The other teeth are very large, with jagged edges, each arising from a common base with one of the groups of teeth already mentioned. The teeth are recurved, so that their ends are directed *forwards*, a feature which is seen in no other subgenus examined.

The *Dorsal Papillæ* are 6 to 8 in number. At the end of the *Anterior Hard Palate* is a single papilla in the middle line, which may be separate from or placed on a narrow extension of the palate.

The *Ventral Papillæ* are present, but are difficult to see, being obscured by the thick chitinisation in this situation.

The *Pigmented Area* is somewhat 'hour-glass' in shape.

The *Posterior Hard Palate* is tongue-shaped, with a constriction near the tip. The posterior portion beyond the pigmented area is covered with small bosses of dark brown chitin, giving the appearance of a 'cobblestone pavement.' This end of the dorsal plate is turned up dorsally to form a massive curved transverse ridge of dark brown, almost black, chitin (Plate XXXIII, fig 28, B)



# EXPLANATION OF PLATE XXXI

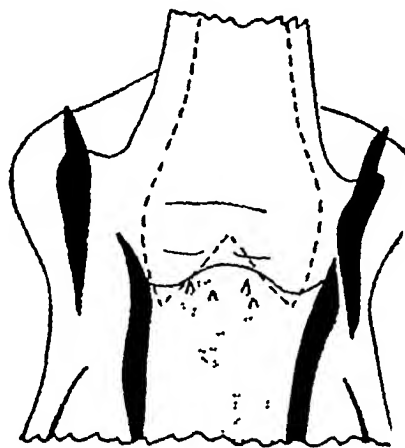
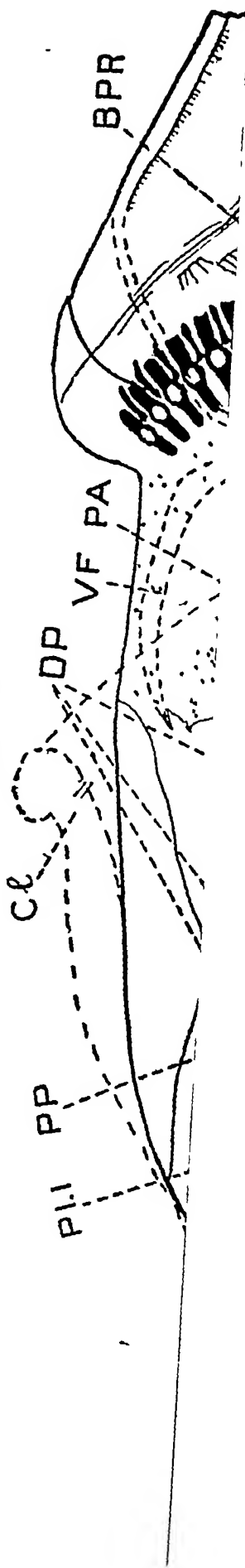
Fig 9	<i>A (Myzomyia) culicifacies</i>	Dorsal view of Buccal Cavity and commencement of Pharynx
, 10	<i>A (Myzomyia) culicifacies</i>	Dorsal view of Bucco-pharyngeal Junction
„ 11	<i>A (Myzomyia) culicifacies</i>	Antero-dorsal view of a separate 'cone' tooth
„ 12	<i>A (Myzomyia) culicifacies</i>	Antero-dorsal view of a separate 'rod' tooth
„ 13	<i>A (Myzomyia) stephensi</i>	Dorsal view of Buccal Cavity and commencement of Pharynx
„ 14	<i>A (Myzomyia) stephensi</i>	Dorsal view of Bucco-pharyngeal Junction
„ 15	<i>A (Myzomyia) stephensi</i>	Antero-dorsal view of a separate 'cone' tooth
„ 16	<i>A (Myzomyia) stephensi</i>	Postero-ventral view of a 'rod' and 'cone'
„ 17	<i>A (Myzomyia) stephensi</i>	Lateral view of a separate 'rod'
„ 18	<i>A (Myzomyia) stephensi</i>	One of the Bucco-pharyngeal ridges, showing the spines which it carries

## EXPLANATION OF LETTERING ON PLATES

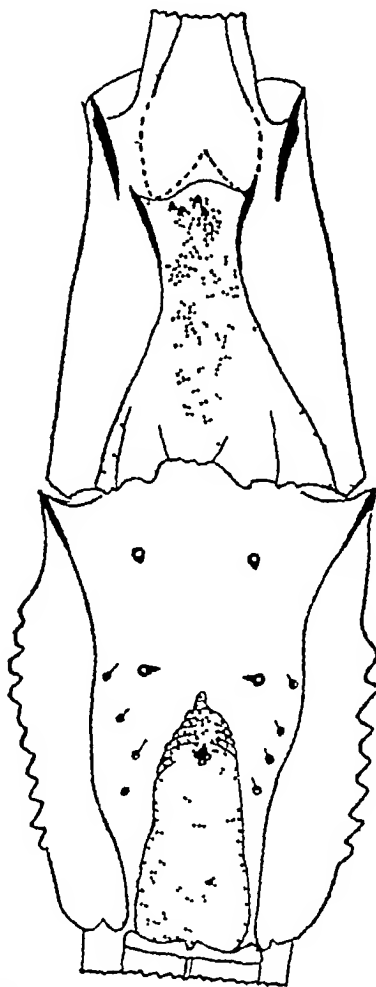
B	Thickened upturned end of ventral plate
BC	Buccal cavity
BPJ	Bucco-pharyngeal junction
BPR	Bucco-pharyngeal ridges with spines
-C	'Cones'
Cl	Outline of clypeus
Cl	Torn edge of clypeus
CS	'Cobblestone' chitimsation
DP	Dorsal papillæ
LF	Lateral flanges
PA	Pigmented area of posterior hard palate
PP	Palatal papillæ
Ph	Pharynx
P1	Anterior hard palate
P2	Posterior hard palate
R	'Rods'
SP	Salivary pump
T	Bucco-pharyngeal armature
VF	Ventral flanges
VP	Ventral papillæ

# EXPLANATION OF PLATE XXXII

- |        |                                  |  |
|--------|----------------------------------|--|
| Fig 19 | <i>A (Myzomyia) pulcherrimus</i> | Dorsal view of Buccal Cavity and commencement of Pharynx |
| „ 20   | <i>A (Myzomyia) pulcherrimus</i> | Dorsal view of Bucco-pharyngeal Junction                 |
| „ 21   | <i>A (Myzomyia) subpictus</i>    | Dorsal view of Buccal Cavity and commencement of Pharynx |
| „ 22   | <i>A (Myzomyia) subpictus</i>    | Dorsal view of Bucco-pharyngeal Junction                 |
| „ 23   | <i>A (Myzomyia) subpictus</i>    | Lateral view of a 'cone' tooth                           |



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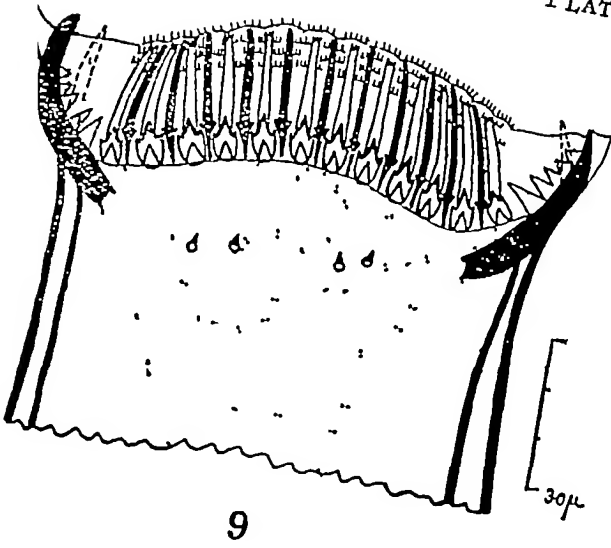
# EXPLANATION OF PLATE XXXIII

- |        |          |   |  |
|--------|----------|---|--|
| Fig 24 | <i>A</i> | ( <i>Myzomyia</i> ) <i>kochi</i>          | Dorsal view of Buccal Cavity and commencement of Pharynx |
| „ 25   | <i>A</i> | ( <i>Myzomyia</i> ) <i>kochi</i>          | Dorsal view of Bucco-pharyngeal Junction                 |
| „ 26   | <i>A</i> | ( <i>Nyssorhynchus</i> ) <i>albimanus</i> | Dorsal view of Buccal Cavity and commencement of Pharynx |
| „ 27   | <i>A</i> | ( <i>Nyssorhynchus</i> ) <i>albimanus</i> | Dorsal view of Bucco-pharyngeal Junction                 |
| „ 28   | <i>A</i> | ( <i>Nyssorhynchus</i> ) <i>albimanus</i> | Posterior end of Posterior Hard Palate                   |
| „ 29   | <i>A</i> | ( <i>Nyssorhynchus</i> ) <i>albimanus</i> | Separate tooth   |

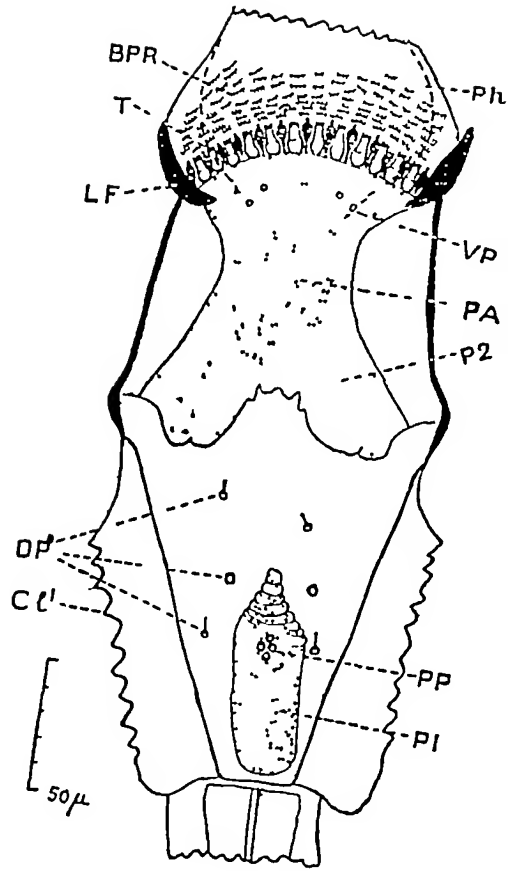
# EXPLANATION OF PLATE XXX

- |     |   |                                   |  |
|-----|---|-----------------------------------|--|
| Fig | 3 | <i>A (Bironella) bironelli</i>    | Dorsal view of Buccal Cavity and commencement of Pharynx |
| „   | 4 | <i>A (Bironella) bironelli</i>    | Dorsal view of Bucco-pharyngeal Junction                 |
| „   | 5 | <i>A (Anopheles) lindesau</i>     | Dorsal view of Buccal Cavity and commencement of Pharynx |
| „   | 6 | <i>A (Anopheles) lindesau</i>     | Dorsal view of Bucco-pharyngeal Junction                 |
| „   | 7 | <i>A (Anopheles) punctumacula</i> | Dorsal view of Buccal Cavity and commencement of Pharynx |
| „   | 8 | <i>A (Anopheles) punctumacula</i> | Dorsal view of Bucco-pharyngeal Junction                 |

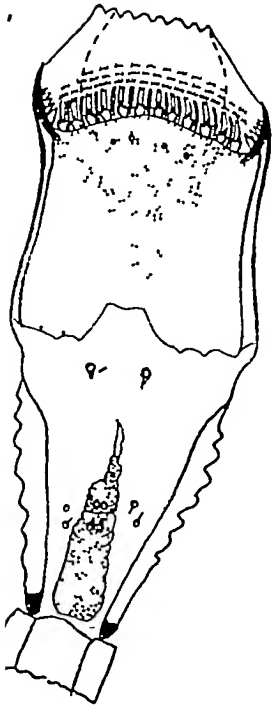




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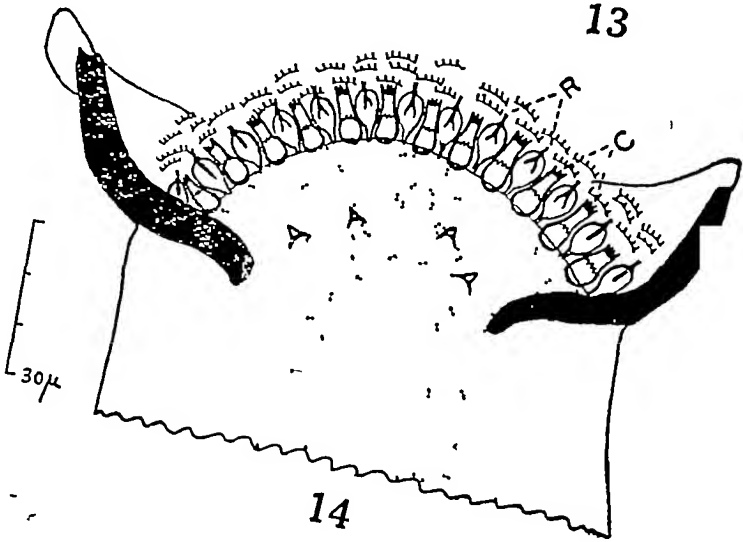
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community Certain villages are inhabited by individuals who have been born and brought up under malarious conditions, and who have acquired immunity against malaria in greater or less degree There are other villages occupied by newly-introduced communities exhibiting the phenomena of acute malarial infestation The labouring convicts, who have for the most part passed many years in the islands, afford material for the study of chronic malaria in the adult, whilst the conditions produced by acute infection in adults may be observed among coolies recently introduced for forest work Kala-azar does not exist in the Andamans, so far as is known, so that the possibility of enlargement of the spleen due to this disease locally acquired is precluded

The time at which these observations were made was towards the end of the malaria season, which was in that year an exceptionally mild one in the Andamans generally

## II METHODS OF INVESTIGATION EMPLOYED

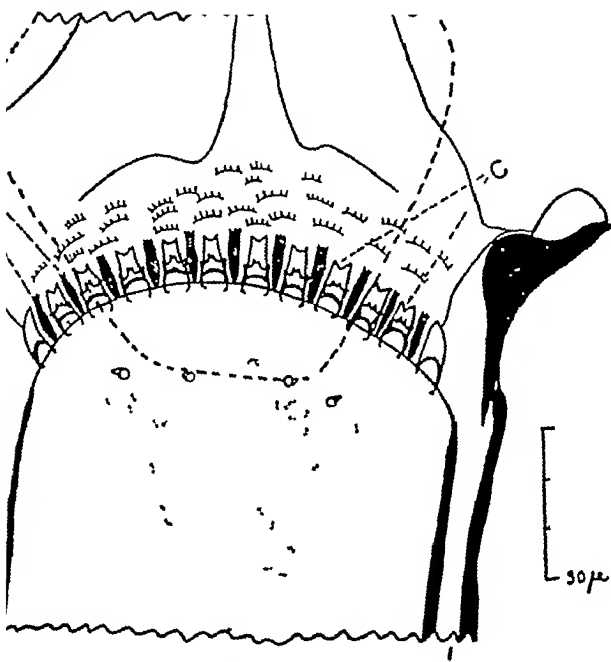
For the purpose of determining the position of the apex of the enlarged spleen in children, the method used was that devised by Christophers (1924*a*), i.e., the distances from the apex of the spleen to the umbilicus, the median line of the body and the costal margin respectively, and the distance from the nipple to the umbilicus, were measured in centimetres with the child standing

In the case of adults, however, it was frequently found difficult to determine the exact position of the apex of the spleen in the erect position, owing chiefly to the greater muscular development of the abdominal wall The men were therefore examined lying down on a perfectly flat surface, such as the floor of a barrack The abdomen was first palpated with the knees drawn up, and if the spleen were found enlarged, the measurements were made with the lower limbs completely extended and the arms by the side of the body in order to secure uniform results At the time of measurement, the man was directed to breathe as quietly as possible, and the measurements were in each case taken at the end of expiration It was found in practice that this procedure actually involved the expenditure of less time than if the men were examined standing, owing to the greater ease with which otherwise 'doubtful' spleens are felt with the subject in the recumbent position

The method used for enumerating the malaria parasites in the blood was that devised by Sinton (1924) In this method, a small quantity of blood from the finger is drawn up to a mark in a pipette and mixed with an equal quantity of a standard suspension of fowl's blood cells The mixture is then blown out on to a slide and made into three thick drops, and the parasites found are counted against the number of fowl's corpuscles observed This method was found a convenient one for application under field conditions In the present case, a suspension containing 9,000 fowl's corpuscles to the c mm was used, and 500 of these were counted during each examination In other words, in each case 0.55 c mm of the patient's blood was examined

## III THE ENLARGED SPLEEN IN ADULTS

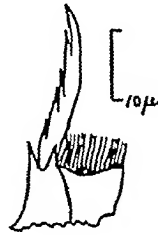
The great majority of the adult spleens measured were those of persons of long residence in the Andamans, suffering from chronic malaria There was one



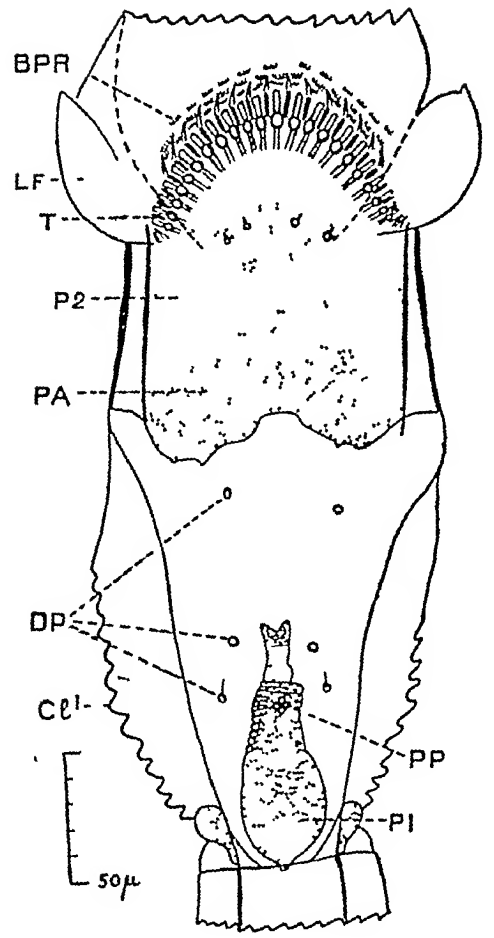
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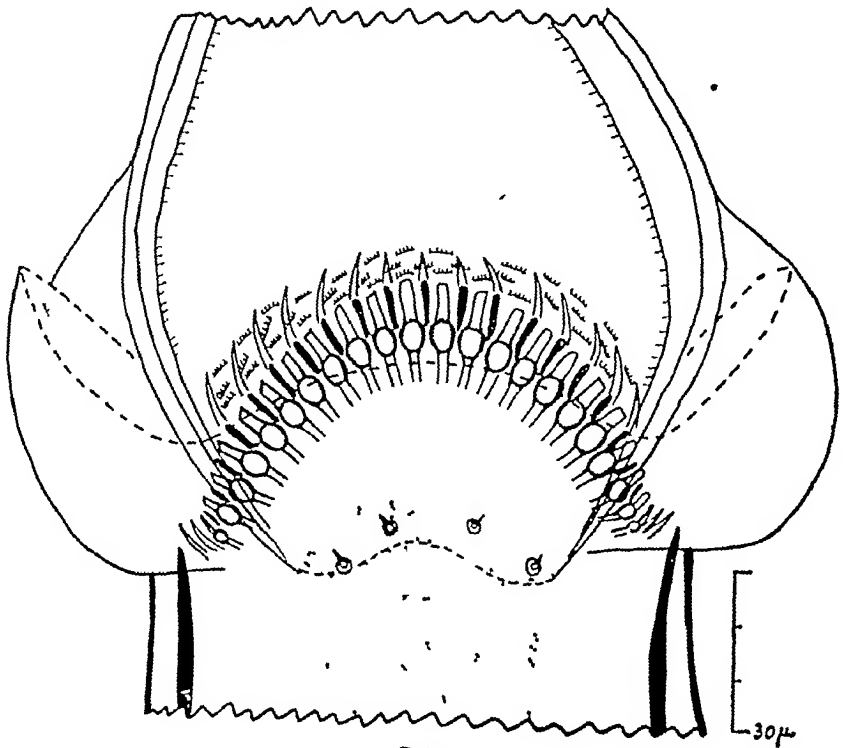
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CHART III.

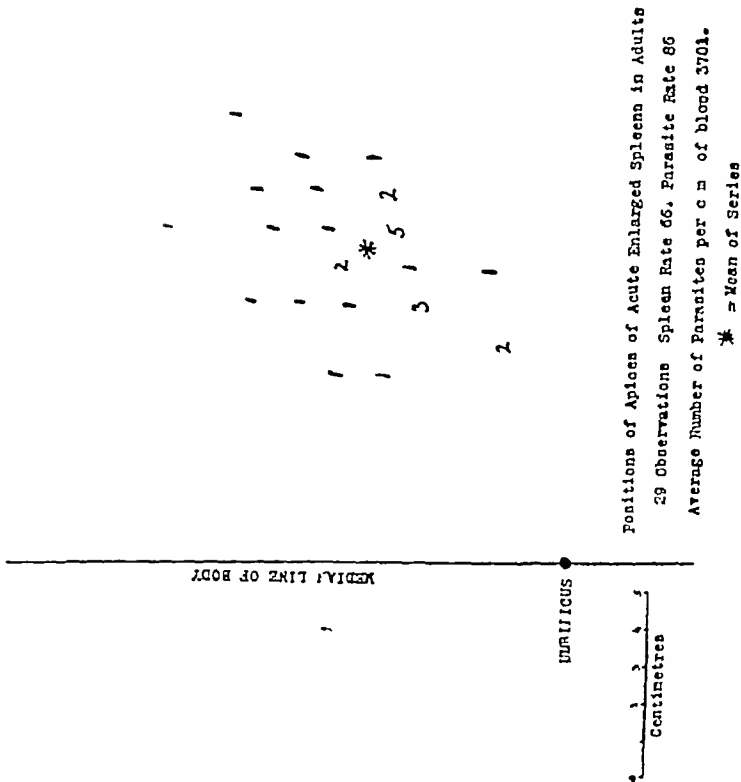
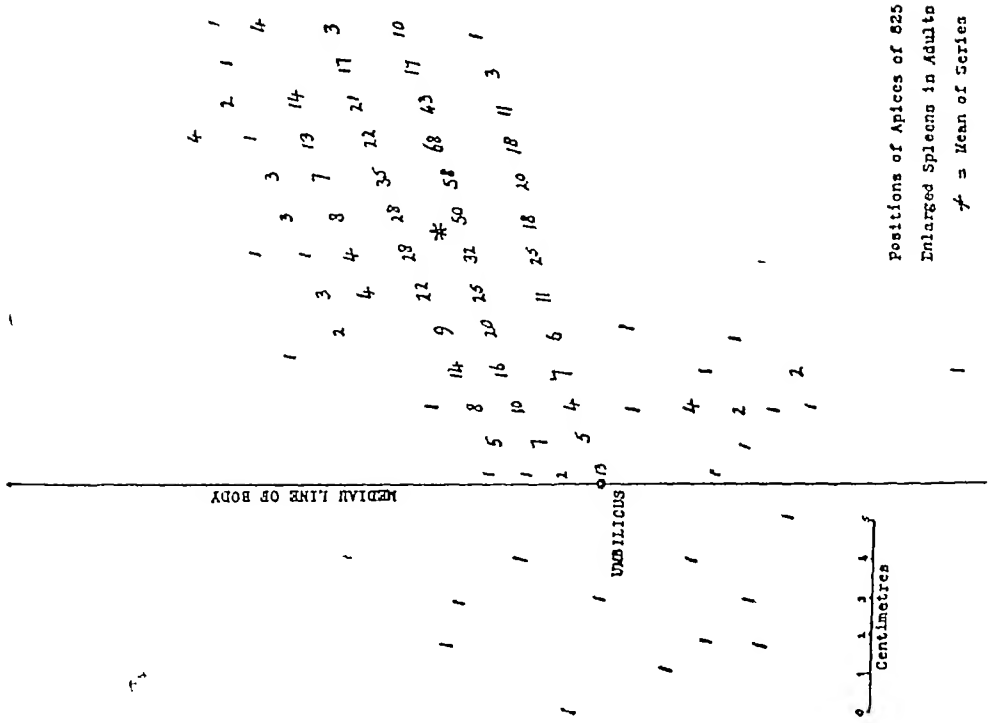
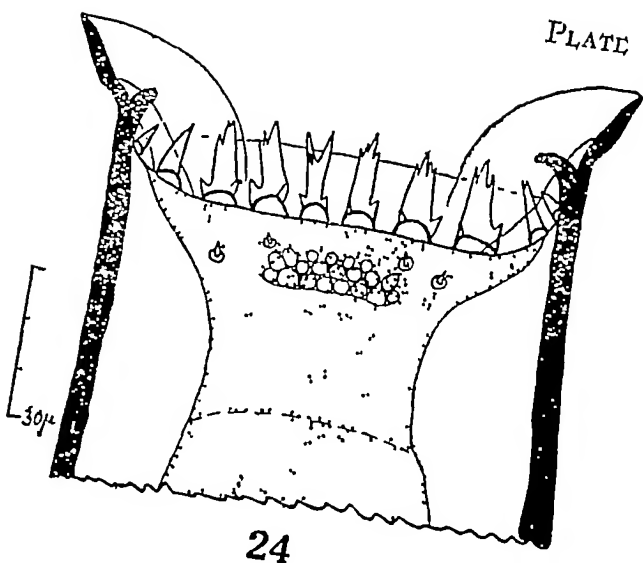
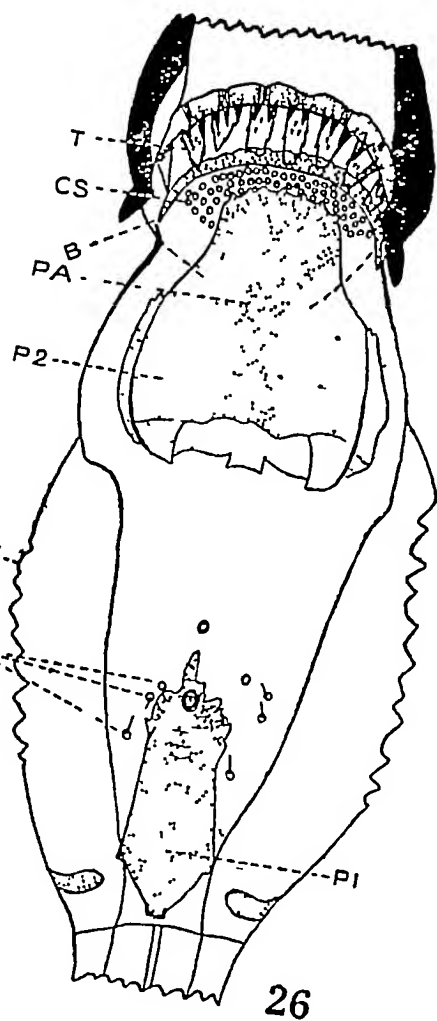


CHART IV

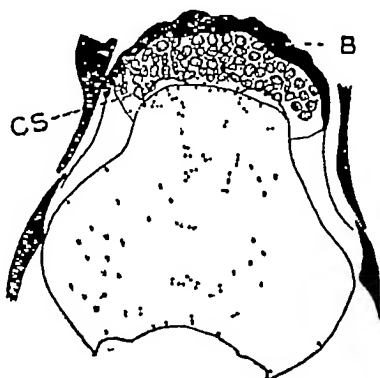




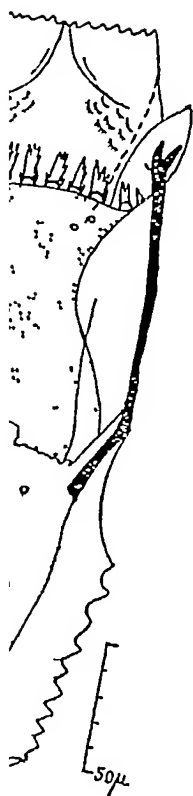
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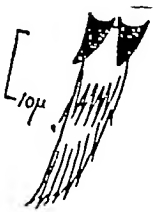
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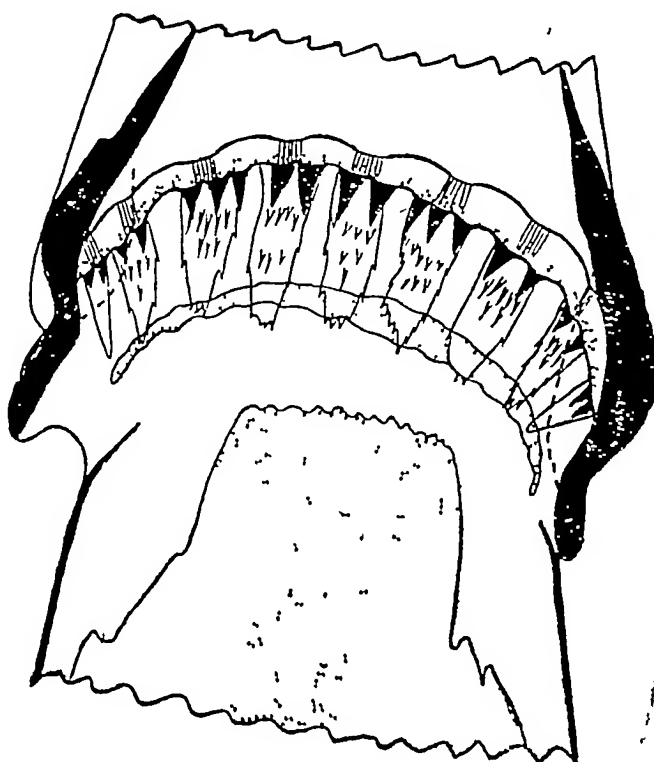


TABLE II

*Actual Measurements of the position of the apex of the enlarged spleen in adults*

CHRONIC SPLEENS, HYPER-ENDEMIC CONDITIONS			CHRONIC SPLEENS, MODERATELY ENDEMIC CONDITIONS			ACUTE SPLEENS, HYPER-ENDEMIC CONDITIONS		
Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations	Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations	Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations
15	12	1	16	12	1	15	12	1
15	11	1	15	15	1			
15	8	1	15	12	2			
14	12	2	14	12	1	14	9	1
14	10	1	14	10	1			
14	9	1	14	9	2			
13	12	3	13	12	7	13	11	1
13	11	9	13	11	7	13	10	1
13	10	5	13	10	8			
12	12	1	13	9	1	12	11	1
12	11	7	12	12	1	12	10	1
12	10	11	12	11	9	12	9	1
12	9	7	12	10	9			
12	8	1	12	9	5			
11	11	2	12	8	2			
11	10	19	11	11	1	11	10	2
11	9	10	11	10	22	11	9	1
11	8	6	11	9	11	11	7	1
11	7	2	11	8	1			
10	10	6	11	6	1			
10	9	26	10	10	5	10	9	5
10	8	7	10	9	37	10	8	2
10	7	4	10	8	16	10	7	1
10	6	1	10	7	3			
9	9	9	9	9	9	9	8	1
9	8	38	9	8	19	9	7	1

# OBSERVATIONS ON MALARIA IN THE ANDAMANS, WITH SPECIAL REFERENCE TO THE ENLARGED SPLEEN IN ADULTS

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## I INTRODUCTION

THE data upon which this paper is based were obtained during a visit to the Andamans in August-September, 1926, for the purpose of inquiring into malarial conditions in the islands. In the course of this enquiry, accurate measurements were made of the position of the apex of the enlarged spleens of 825 adults and 240 children, and parasite counts were made in the case of 208 positive blood films. All the spleen measurements were made and all the blood films taken by one of us (G C) personally, so that although the numbers of observations are small, the results obtained are strictly comparable one with another.

A method for the accurate measurement of the position of the spleen was introduced by Christophers (1924*a*). This consists in the triangulation of the apex, or most projecting part, of the spleen by measurements from the umbilicus and from the median line of the body, and is far more accurate than the old method of estimating its enlargement in fingers' breadths. Christophers has published the results of an exhaustive analysis of observations made on the blood and spleens of children during the course of malarial surveys made at Singhbhum, Chota Nagpur, and at Nalbari and Nongpoh, Assam (Christophers, 1924*a*, 1924*b*, 1924*c*, 1925). More recently, MacDonald (1926) has applied Christophers' methods in the examination of children in Freetown, Sierra Leone.

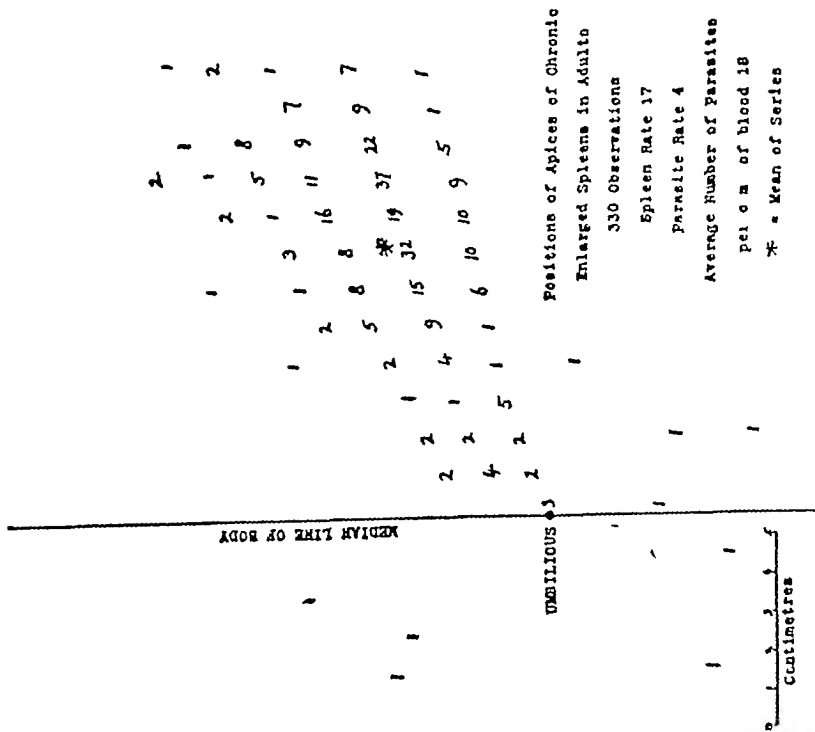
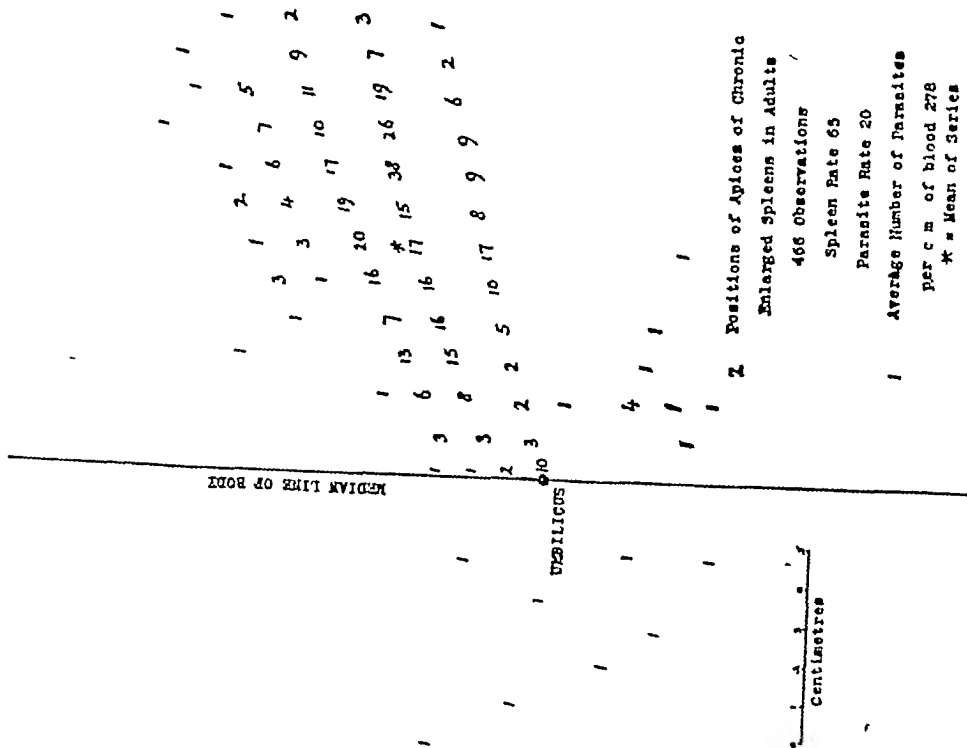
So far as we are aware, no observations of a similar nature have hitherto been published with regard to the measurement of the enlarged spleen in adults.

Conditions in the Andamans are peculiarly favourable for the study of malaria. In the first place, the disease is localised, parts of the islands being highly malarious, whilst others are almost or entirely free from malaria, depending on the presence or absence of *A ludlowi*, which is the only malaria carrier of any importance in the islands (Christophers, 1912, Covell, 1927). Secondly, there are unusual opportunities for studying malarial conditions among different types of

TABLE II—(concl'd)

CHRONIC SPLEENS, HYPER-ENDEMIC CONDITIONS			CHRONIC SPLEENS, MODERATELY ENDEMIC CONDITIONS			ACUTE SPLEENS HYPER-ENDEMIC CONDITIONS		
Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations	Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations	Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations
3	1	3	3	1	2			
3	0	1						
3	-2	1						
3	-3	1						
2	2	2	2	2	2			
2	1	3	2	1	4			
2	0	1						
1	1	3	1	1	2			
1	0	2						
0	0	10	0	0	3			
-2	1	1						
-3	2	4	-3	0	1			
-3	-2	1						
-4	1	1	-4	2	1			
-4	2	1	-4	4	1			
-4	3	1						
-5	2	1	-5	-1	1			
-5	4	1						
-5	-2	1						
-5	-4	1						
-5	-5	1						
-6	3	1	-6	2	1			
-7	6	1	-6	-4	1			
-10	3	1						

Note—A minus sign in the apex-umbilicus column denotes a measurement below the umbilicus. A minus sign in the apex-median line column denotes a measurement to the left of the median line.





that the average weight of that organ in persons dying in the settlement is about 2½ times that of the normal, whilst in the case of those whose deaths are attributed directly to malaria it is nearly 4 times that of the normal. Out of 4,474 adults examined in the settlement, 1 372, or 31 per cent, were found to have enlarged spleens. Accurate measurements of the position of the apex of the spleen in 796 of these gave a mean position situated 8 cms from the umbilicus, with an average costal projection of 5.4 cms.

#### V MALARIAL INFECTION IN NEWLY-INTRODUCED AND LOCAL-BORN COMMUNITIES UNDER HYPER-ENDEMIC CONDITIONS

In a village called Muthura there was living a newly-introduced community of Bhatus, who had been brought from the United Provinces, India, six months before my visit. This village was situated within 400 yards of a salt swamp, which formed a breeding-place for *A. ludlowi*, specimens of which were caught in the huts. They had received a certain amount of quinine, which was administered, probably in a somewhat irregular fashion, by the village chowkidar. Nevertheless, as the result of the investigations made there show, they present an interesting picture of a community living under 'attack' conditions.

As a contrast to these will be studied the figures relating to the inhabitants of two old-established villages, Bumtitan and Taylerabad, situated in the hyper-endemic area. The children in this case were all born in the villages, and the adults had for the most part either spent all their lives there, or had lived there for periods carrying from 5 to 20 years. The ages of all the children examined were between two and ten years, so that they were past the stage of 'acute infestation' described by Christophers (1924c).

The figures relating to the two communities in question are given in Table IV.

TABLE IV

*Spleen and Parasite Findings among Children in Newly-introduced and Local-born Communities\**

	Number examined for enlarged spleen	Number with spleen enlarged	Spleen rate	Average apex-umbilicus measurement in cms	Average apex-median line measurement in cms	Average costal projection in cms	Number of bloods examined	Number found infected	Percentage found infected	Average parasites per c.mm of blood in those infected
Newly-introduced population	64	49	77	9.2	6.8	3.8	71	43	60	1,277
Local-born population	45	30	67	8.3	6.1	4.8	40	17	42	229

\* The measurements relating to the position of the apex of the spleen in children have been corrected according to the correction-table given by Christophers and Khazan Chand (1924).

community, however, which presented the picture of acute malarial infection in adults. This consisted of a party of Karen coolies recently introduced from Burma, who were employed in felling timber on a small island off Middle Andaman. Their camp had been established on the island for about three months, and the men were suffering severely from malaria. About half of them actually had raised temperatures at the time of my visit. All the anopheline mosquitoes captured in the camp were *A. ludlowi*, and 97 of these were caught in a single hut. It is of course possible that some of the men had enlarged spleens before leaving Burma, though according to their own statements they had suffered very little from fever in their own country. The parasite counts show that at the time of examination, they were under 'attack' conditions.

The adults suffering from chronic enlargement of the spleen fall into two categories, namely, those living under hyper-endemic conditions, with a spleen rate of 65 per cent and those living under conditions of moderate endemicity, with a spleen rate of 17 per cent.

Table I shows the spleen rate, parasite rate, average parasite value and mean measurements regarding the position of the apex of the enlarged spleen in the three classes of adults under consideration. The actual positions of the apices of the enlarged spleens with relation to the umbilicus and the median line of the body in the three classes are set out in Charts I, II and III, whilst the corresponding measurements in centimetres are shown in Table II. Chart IV gives the positions of the apices of all the adult spleens measured. In each Chart, the position of the mean of the series is indicated by a star.

TABLE I  
*Spleen and Parasite Rates in Adults*

	Number examined	Number with enlarged spleen	Spleen rate	Parasite rate	Average parasites per c.mm of blood	Mean apex-umbilicus measurement in cms	Mean apex-median line measurement in cms	Mean costal projection measurement in cms
Chronic malaria, hyper-endemic conditions	712	466	65	20	278	7.4	6.3	6.0
Chronic malaria, moderately endemic conditions	1,983	330	17	4	18	8.7	7.5	4.5
Acute malaria, hyper-endemic conditions	44	29	66	86	3,701	10.0	8.4	3.1
TOTAL	2,739	825	30			8.0	6.8	5.2

In the case of chronic infections in adults the percentage of infections increases as the spleen enlarges, as is the case in the other two series. As regards the parasite value it is dangerous to draw deductions from a series as small as this, for it is obvious that a single individual undergoing an acute attack will raise the average value for the group concerned in a disproportionate degree. In this series there were only two infections with a parasite value of over 1,000 per c mm. In one of these, the spleen was not palpable, whilst in the other, the apex was situated 12 cms from the umbilicus. As far as the figures go, they would appear to indicate that the highest parasite values are associated with spleens of moderate enlargement, the value decreasing with great enlargement of the organ.

TABLE V  
*Relation of Enlargement of Spleen to Infection—Children \**

Apex-umbilicus measurement in cms	Number of observations	Average costal projection in cms	Number found infected	Percentage found infected	Number of parasites per c mm in each case	Total parasites found	Average parasites per c mm of blood in those infected
Not palpable	414		57	11	17,190—5,382—3,936—3,744—2,354—990—918—810—648—576—558—522(3)—486—484—450—432—414—396(2)—370—342—306—270—252(3)—234—216—180—162(3)—126(5)—90(5)—72—54(3)—36(4)—18(4)—16	45,976	806
14—12	51	1	20	39	4,590—2,070—1,944—1,160—1,008—990—918—486—346—342—270(3)—234—198—162—126—72(2)—8	18,536	926
11—8	90	3.5	39	43	9,000—5,760—4,050(2)—2,844—2,700—2,160—2,070—1,980—1,530—1,314—1,098—480—450(2)—318—288—270(2)—216(4)—126(3)—108(2)—90(5)—54(2)—36—18(3)	43,284	1,109
7—4	34	7.3	17	50	9,000—6,300—2,630—1,496—990(2)—810—594—396—378—334—200—152—126(2)—90—18	24,610	1,448
3—0	3	10.3	3	100	202—180—72	454	151
Beyond Umbilicus	1	13	1	100	252	252	252
TOTAL	593		137	23		133,112	971

\* Measurements relating to the position of the apex of the spleen in children have been corrected according to the correction-table given by Christophers and Khazan Chand (1924)

TABLE II—(contd)

CHRONIC SPLEENS, HYPER-ENDEMIC CONDITIONS			CHRONIC SPLEENS, MODERATELY ENDEMIC CONDITIONS			ACUTE SPLEENS, HYPER-ENDEMIC CONDITIONS		
Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations	Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations	Apex-umbilicus measurement in cms	Apex-median line measurement in cms	Number of obser- vations
9	7	13	9	7	8			
9	6	3	9	6	1			
9	5	3						
9	3	1						
8	8	9	8	8	10	8	8	1
8	7	15	8	7	32	8	7	3
8	6	20	8	6	8	8	5	1
8	5	1	8	5	2			
8	4	1	8	4	1			
8	—7	1	7	7	10	7	5	1
7	7	8	7	6	15			.
7	6	17	7	5	5			.
7	5	16	7	—2	1			
6	6	17	6	6	6	6	6	2
6	5	16	6	5	9			
6	4	7	6	4	2			
6	—6	1	6	—4	1			
5	5	10	5	5	1			.
5	4	16	5	4	4			.
5	3	13	5	3	1			..
5	2	1	5	—3	1			
4	4	5	4	4	1			
4	3	15	4	3	1			
4	2	6	4	2	2			
3	3	2	3	3	5			
3	2	8	3	2	2			

## VII THE OUTPUT OF CRESCENTS IN RELATION TO THE PARASITE COUNT

Out of 122 blood films in which malignant tertian parasites were found, crescents were present in 76, or 62 per cent. The relative proportions in which they were found in acute and chronic infections in children and adults are shown in Table VIII. All children under the age of two years are included under the category of 'acute' infections.

TABLE VIII  
*Figures dealing with the Output of Crescents*

	Number examined	Number of crescent infections	Percentage of crescent infections	Total number of crescents found.	Average value of crescent infections
Acute infections (children)	74	25	34	7,434	297
Acute infections (adults)	44	15	34	4,858	324
Chronic infections (children)	519	27	5	4,060	150
Chronic infections (adults)	200	9	4	1,052	117

Among the children suffering from acute infection the highest crescent infection encountered was 1,782 per c mm. The average of the asexual infections associated with the presence of crescents was 1,548. Collectively in this group of children there were 7,434 crescents to 36,992 asexual forms, or about 1 to 5.

Among the children with chronic infection the highest crescent infection encountered was 522 per c mm. The average of the asexual infections associated with the presence of crescents was 458. Collectively in this group of children there were 4,060 crescents to 13,888 asexual forms, or about 1 to 3.

Among the adults suffering from acute infection the highest crescent infection encountered was 1,800 per c mm. The average of the asexual infections associated with the presence of crescents was 4,604. Collectively in this group of adults there were 4,858 crescents to 48,064 asexual forms, or about 1 to 10.

Among the adults with chronic infection the highest crescent infection encountered was 450 per c mm. There were no asexual infections associated with the presence of crescents. Collectively in this group of adults there were 1,052 crescents to 3,736 asexual forms, or about 1 to 4.

These findings are in agreement with those of Schuffner (1920) and of Christophers (1924c), namely, that the formation of crescents is not associated with immunisation, but is reduced during this process, and that the infectivity of a community would appear to depend upon the presence of acute infections and

A comparison of the figures relating to the three classes of community under consideration shows that in the case of chronic enlargement of the spleen the average size of the enlarged organ is considerably greater among those with a high spleen rate than among those living in the moderately endemic area. In the case of individuals suffering from acute enlargement of the spleen the condition is what one would expect from the observations made by Christophers. That is, there is a high spleen and parasite rate, with a high average parasite count, but the average enlarged spleen is considerably smaller than is the average 'chronic' enlarged spleen, even among a community with a spleen rate of only 17 per cent.

In Chart IV is shown the position of the apices of all the enlarged adult spleens accurately measured. An analysis of the figures relating to 128 adult spleens of which the apices were situated at a distance of 1 cm or less from the costal margin shows that the mean position for spleens which are just palpable lies at a point approximately 12 cms from the umbilicus, and 10 cms from the median line of the body. If a line be drawn from this point to the umbilicus it passes through the mean position of the apex in the case of each of the three communities under consideration, and also through the mean position of the apex for all the adult spleens measured. Out of the 825 spleens measured, 695 or 84 per cent, of the apices lie within 2 cms distance from this line, 396 of these lying at a distance of from 6 to 11 cms from the umbilicus. It would appear that the normal path of enlargement of the spleen in adults follows approximately the same line as that indicated by Christophers in the case of enlarged spleens in children.

#### IV THE AVERAGE WEIGHT OF THE ADULT SPLEEN POST-MORTEM IN THE ANDAMANS

The following Table (Table III), which shows the average weights of the spleen recorded at post-mortem examinations held in the settlement of Port Blair from 1917 to 1926, together with the cause of death, may be of interest. Taking the average weight of the normal adult spleen to be about 7 ounces, it will be seen

TABLE III  
*Average Weight of Adult Spleens Post-mortem*

Cause of death	Number of cases	Average weight of spleen in ounces
Malaria	54	27.4
Influenza	5	25.8
Cirrhosis of liver	12	24.8
Blackwater fever	8	22.9
Pneumonia and its complications	126	21.1
Jaundice	12	15.2
Dysentery	82	13.6
Phthisis	53	12.6
Other causes	177	17.6
TOTAL	529	18.6

TABLE X  
*Relation of Species of Parasite to Size of Spleen*

Species of parasite	ADULTS		CHILDREN		
	Number of observations	Average apex-umbilicus measurement in cms	Number of observations	Percentage with enlarged spleen	Average apex-umbilicus measurement in cms
M T	22	7.7	50	60	9.4
B T	5	7.2	5	42	7.8
Quartan	23	8.6	21	60	8.6

The number of observations is of course too few to enable any precise conclusions to be drawn. In both children and adults the average enlarged spleen in the case of B T infections were the largest of the three. On the other hand the spleen rate in the case of B T infections was only 42, compared with a rate of 60 in the case of both M T and quartan. Among the adults the average enlarged spleen in quartan infections was the smallest of the three, whilst in children the size was intermediate between that encountered in B T and M T infections.

As far as these few results go, they appear to indicate that there is very little difference in the effect of the different species of parasite on the enlargement of the spleen.

### CONCLUSIONS

1 The results of 825 observations on the position of the apex of the enlarged spleen in adults as determined by accurate measurement are analysed.

2 The normal path of enlargement of the spleen in adults does not appear to differ materially from that observed among children.

3 In the series dealt with the position of the apex of the spleen in the case of 84 per cent of the individuals examined lay within 2 cms. of a line drawn from the umbilicus to a point 12 cms. distant from it, and 10 cms. from the median line of the body. The mean position of the apex in a series of acutely enlarged spleens, in a series of chronic enlarged spleens occurring in a moderately endemic area, and in a series of chronic enlarged spleens occurring in a hyper-endemic area all lay approximately upon this line.

4 In the case of chronic infections, an increased spleen rate among adults is associated with a greater size of the average enlarged spleen.

5 In the case of both adults and children the percentage of parasite infections increases as the spleen enlarges.

6 The average parasite value increases with the size of the spleen up to a certain degree of enlargement. The size of spleen associated with the highest parasite value in adults was one with the apex situated at a distance of about 6.8 cms. from the umbilicus, corresponding with an average costal projection of

In both communities the spleen rate was high, but in the newly-introduced community the average size of the enlarged spleen was less than in the case of the local-born, whilst the parasite rate and the average parasite value was much greater. The latter would no doubt have been greater still, had not a certain amount of quinine been administered. In the newly-introduced community there were 12 infections of over 1,000 parasites per c mm, or 17 per cent of those examined, whilst 21 children (30 per cent) showed infection with crescents, the average value of crescent infections being 240 per c mm. In the local-born community there was only one infection of over 1,000 parasites per c mm, or 2.5 of those examined, whilst 6 children (15 per cent) showed crescent infections, with an average value of 166 per c mm.

As regards the adults, in the old-established villages there was a spleen rate of 50 per cent, a parasite rate of 15 per cent, and an average parasite value of 325 per c mm, but in only one case was there a count of over 1,000 per c mm. There were 5 per cent, of crescent infections, with an average value of 176 per c mm.

In the case of the newly introduced community the adult spleen rate was 75 per cent. No adults of this group were examined for parasite infections.

These results are in accordance with what one would expect from the researches of Christophers into the subject of immunity against malaria under hyper-endemic conditions.

#### VI THE RELATION OF ENLARGEMENT OF THE SPLEEN TO INFECTION

In Tables V, VI and VII are given the average number of parasites per c mm found in association with spleens of different degrees of enlargement. The figures relating to (a) children, (b) adults suffering from acute infection, and (c) adults suffering from chronic infection, are shown separately.

Christophers, in his Singhbhum series, found that in children a high parasite value was associated with spleens of such a size that the apex lay about 8-10 cms. from the umbilicus, the corresponding costal projection being about 5 cms, or 'three-finger' spleens. He also found that the parasite value with spleens of greater size was not increased, but was diminished.

In the present series the findings as regards the children are substantially in agreement with those of Christophers. The highest parasite value is associated with spleens of which the apices lie about 6-10 cms from the umbilicus, corresponding with an average costal projection of about 5 cms whilst there is a marked drop in the parasite value as the apex approaches the umbilicus. The percentage of individuals infected, however, increases progressively with the enlargement of the organ.

In the case of acute infections in adults, the percentage of infections also increases progressively as the spleen enlarges. The parasite value in spleens not palpable is only moderately high, the value increasing rapidly as the spleen enlarges. In this series the community in question had been living in the highly malarious area for only about three months, and there were no spleens of a size greater than is represented by an apex-umbilical measurement of 6 cms, so that it is impossible to say whether or not the parasite value tends to decrease with spleens of greater size than this.



10 SINTON, J A (1924) Methods for the Enumeration of Parasites and Leucocytes in the Blood of Malaria Patients *Ind Jour Med Res*, XII, 2, pp 341—346

11 SWELLENGREBEL, N H, SCHUFFNER, W and SWELLENGREBEL DE GRAAF, J M H (1919) The susceptibility of Anophelines to Malaria Infections in Netherlands India *Meded v d Burg Geneesk D in Nederlandsch, Indie*, Anno 1919, Deel, III, pp 1—64

TABLE VI

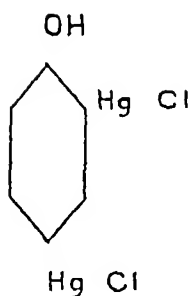
*Relation of Enlargement of Spleen to Infection—Acute Malaria in Adults*

Apex-umbilicus measurement in cms	Number of observations	Average costal projection in cms	Number found infected	Percentage found infected	Number of parasites per c mm in each case	Total parasites found	Average parasites per c mm of blood in those infected
Not palpable	15		11	73	3,042—2,016 864—720—702—432—420—162 92—90—54	8,594	781
15—12	7	0.7	6	85	22,500—4,596—1,602—1,044 252 90	30,196	5,024
11—9	14	2.7	13	92	12,980—10,242—8,334—5,986— 3,942—3,582—2,790—1,800— 1,782—1,062 810 90—54	53,474	4,113
8—6	8	6.2	8	100	21,240—11,893—5,868—4,590— 3,420—1,098 432—162	48,698	6,087
TOTAL	44		38	86		140,912	3,708

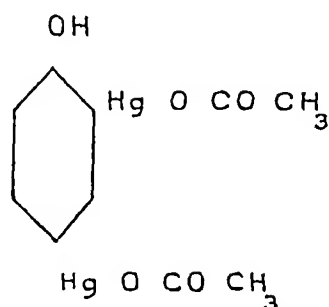
TABLE VII

*Relation of Enlargement of Spleen to Infection—Chronic Malaria in Adults*

Apex-umbilicus measurement in cms	Number of observations	Average costal projections in cms	Number found infected	Percentage found infected	Number of parasites per c mm in each case	Total parasites found	Average parasites per c mm of blood in those infected
Not palpable.	50		7	14	3,600 54—36(4)—18	3,816	545
15—12	22	1.8	4	18	2,790 108—72—18	2,988	747
11—8	79	3.8	10	12	450—180—72(3)—54—18(4)	972	97
7—4	29	7.1	4	14	54(2)—36—18	162	40
3—0	14	10.5	5	36	180—72—54—50—18	354	71
Beyond umbilicus	6	16.6	2	33	52—50	102	51
TOTAL	200		32	16		8,394	262



o, p-dichloromercuriphenol



o, p-diacetoxymercuriphenol

Owing to the presence of the nuclear hydroxyl these substances are soluble in caustic soda, and their bactericidal concentration was determined in alkali solution.

*o, p-diacetoxymercuriphenol*—Phenol and two moles of mercuric acetate were heated on the steam bath, and the product dissolved in dilute acetic acid. The mercurated phenol crystallised in minute white needles which melted at  $216.7^{\circ}$ .

*p-chloromercuriphenol*—The mother liquor from the diacetoxymercuriphenol was saturated with sodium chloride, and the resulting precipitate extracted with cold alcohol. The portion insoluble in cold alcohol crystallised from boiling alcohol in fine needles melting at  $220^{\circ}$ .

*o-chloromercuriphenol*—The filtrate from the para compound was evaporated on the water bath, and the ortho chloride crystallised from hot water in minute needles melting at  $152^{\circ}$ .

*o, p-dichloromercuriphenol*—An aqueous solution of phenol and two moles of mercuric acetate were heated on the water bath, and the product treated with sodium chloride. The resulting precipitate was extracted with boiling alcohol, when the dichloride was left as an insoluble white powder which decomposed at  $258^{\circ}$  without melting. This substance dissolved in  $\frac{N}{1}$  sodium thiosulphate, but slowly decomposes on standing.

TABLE I

	BACTERICIDAL CONCENTRATIONS		Mercury per cent (app.)
	15 minutes contact	24 hours contact	
Phenol	1 600	1 600	
2, 4, 6-trichlorophenol	1 2000	1 10000	
o-chloromercuriphenol	1 320000	1 2560000	61
p-chloromercuriphenol	1 320000	1 5120000	61
o, p-dichloromercuriphenol	1 320000	1 1280000	71
o, p-diacetoxymercuriphenol	1 160000	1 640000	(unstable) 65

not directly on the evidence of intensity given by the amount of splenic enlargement

# VIII THE RELATIVE PROPORTION OF THE DIFFERENT SPECIES OF MALARIA PARASITE IN THE ANDAMANS

Out of 837 blood examinations made, malaria parasites were found in 208. Mixed infections were encountered in 10 cases. The relative proportions of the different species of parasite are shown in Table IX.

TABLE IX  
*Relative Proportions of the Different Species of Parasite*

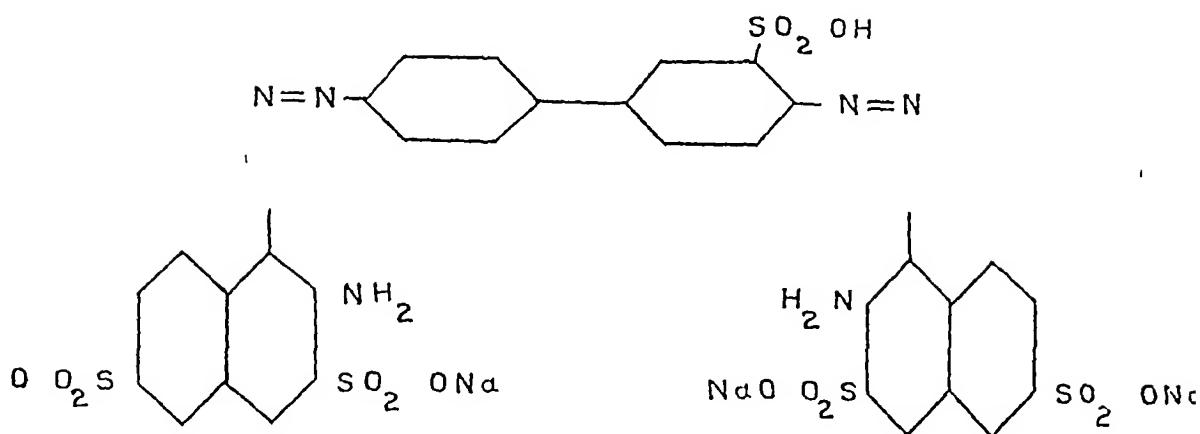
	Number found infected	M T		B T		QUARTAN	
		Number of infections	Percentage to total infections	Number of infections	Percentage to total infections	Number of infections	Percentage to total infections
Children	137	90	65	13	9	40	29
Adults	71	32	45	10	14	33	46
TOTAL	208	122	59	23	11	73	35

The comparatively high proportion of quartan infections in the Andamans was noted by Christophers in 1912. He suggested that this might be due to the fact that the majority of his cases were probably relapses, and that the parasite of quartan malaria is more persistent than the other two species. In the case of the results tabulated above, however, the high proportion of quartan infections was just as apparent in acute infections as in chronic. For instance, in the case of the Karen coolies in Middle Andaman, out of 38 cases in which parasites were found there were 19 infections with M T parasites, 6 with B T, and 17 with quartan.

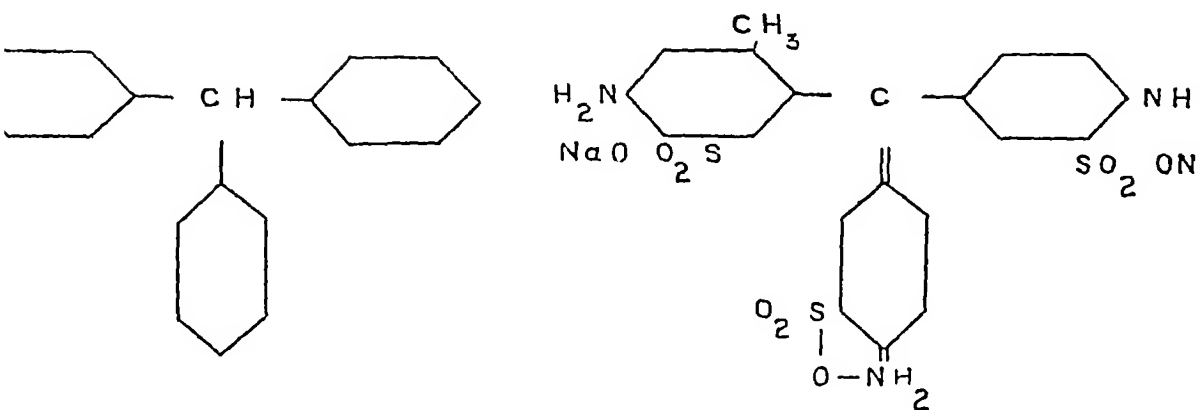
Swellengrebel, Schuffner and Swellengrebel de Graaf (1920) showed that under experimental conditions *A. ludlowi* can be infected with quartan parasites, but that its susceptibility to this species is apparently very much less than is the case with M T and B T parasites. It appears that there must be some hitherto unexplained factor or factors which lead to the comparatively high incidence of quartan malaria in the Andamans.

# IX THE RELATION OF SPECIES OF PARASITE TO SIZE OF SPLEEN

It is sometimes stated that the quartan parasite is associated with a greater degree of splenic enlargement than are the other two species. An attempt was made to see if the presence in the blood of the different species of parasite were associated with different degrees of enlargement of the spleen in the Andamans. The results of this investigation are shown in Table X, mixed infections being excluded.



Trypan Red



Triphenylmethane

Acid Fuchsin

A solution of mercuric acetate was prepared by dissolving one or more moles of the salt in water, filtering, and adding two drops of glacial acetic acid to the filtrate. The filtrate was poured gradually and with constant stirring into water holding one mole of the dye in solution. The mixture was then concentrated on the water bath and dried *in vacuo* over calcium chloride.

6.2 cms In children the highest parasite value was found in the case of spleens with the apex situated at a distance of about 6-11 cms from the umbilicus, with an average costal projection of 5 cms. These measurements correspond approximately with a 'three-finger' spleen in children, and a 'three to four-finger' spleen in adults. Spleens of a greater size than these are associated with a progressively decreasing parasite value.

7. As regards the output of crescents, the findings of Schuffner and of Christophers are confirmed, namely that the gamete output is not associated with immunisation, but the reverse.

8. There is in the Andamans an unusually high proportion of quartan infections. This high proportion is found in association with acute as well as with chronic infections. The reason for this is not explained.

9. So far as can be judged from the comparatively few figures available, there does not appear to be any marked difference between the degree of enlargement of the spleen associated with the different species of malaria parasite.

10. The exact methods of measuring the position of the apex of the enlarged spleen devised by Christophers are capable of application in the field in the case of adults, with certain modifications, and give far more accurate results than those formerly employed.

11. Sinton's method of enumerating malaria parasites by means of a standard suspension of fowl's blood is found to be convenient and easy of application under field conditions.

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TABLE II

		BACTERICIDAL CONCENTRATIONS		Mercury per cent ( app )
		15 minutes contact	24 hours contact	
Trypan Blue		nil	nil	
mono-acetoxymercuri-Trypan Blue		1 9600	1 64000	16
di-                   "                   "		1 19200	1 128000	27
tri-                   "                   "		1 64000	1 256000	35
tetra-               "               "		1 64000	1 358400	40
penta-              "              "		1 64000	1 256000	44 *
hexa-               "               "		1 64000	1 153600	48 *
hepta-             "             "		1 64000	1 153600	50 *
octo-               "               "		1 38000	1 153600	53 *
Trypan Red		nil	nil	
mono-acetoxymercuri-Trypan Red		1 22400	1 76800	16
di-                   "                   "		1 38400	1 153600	26
tri-                   "                   "		1 64000	1 256000	34
tetra-               "               "		1 76800	1 358400	39
penta-              "              "		1 51200	1 153600	44 *
hexa-               "               "		1 38400	1 230400	47 *
hepta-             "             "		1 44800	1 115200	50 *
octo-               "               "		1 51200	1 153600	53 *
Acid Fuchsin		nil	nil	
mono-acetoxymercuri-Acid Fuchsin		1 14400	1 51200	23
di-                   "                   "		1 32000	1 128000	36
tri-                   "                   "		1 32000	1 179200	44
Mercurochrome-220-soluble		1 3200	1 102400	26

\* Mercury partly thrown out of solution

## SUMMARY

Mercurial derivatives of Phenol, Trypan Red, Trypan Blue, and Acid Fuchsin have been prepared and their bactericidal action on *B. pestis* has been determined *in vitro* by the so-called 'inhibitory method'

Aromatic mercurials are many times more active than the compounds they are derived from

# THE BACTERICIDAL ACTION OF SOME ORGANIC COMPOUNDS OF MERCURY ON *BACILLUS PESTIS*

BY

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AND

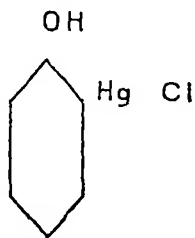
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[Received for publication, May 23, 1927]

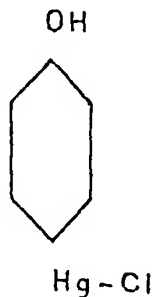
THE mercurial compounds discussed in this paper all belong to that group of compounds in which one valency of the mercury atom is satisfied by an organic radical. They may be divided into two classes (1) compounds derived from phenol, and (2) compounds derived from dyestuffs.

## I *Compounds derived from phenol*

The four compounds of this class result from the mercuration of phenol and are characterized by the presence of a nuclear hydroxyl, and by the mercury being linked to the nuclear carbon through one valency and to either an atom of chlorine or to an acetoxy group through the other.



o-chloromercuriphenol



p-chloromercuriphenol



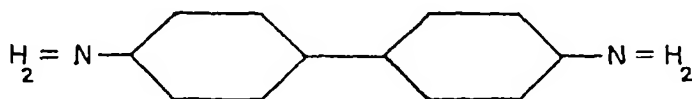


As may be seen from Table I —

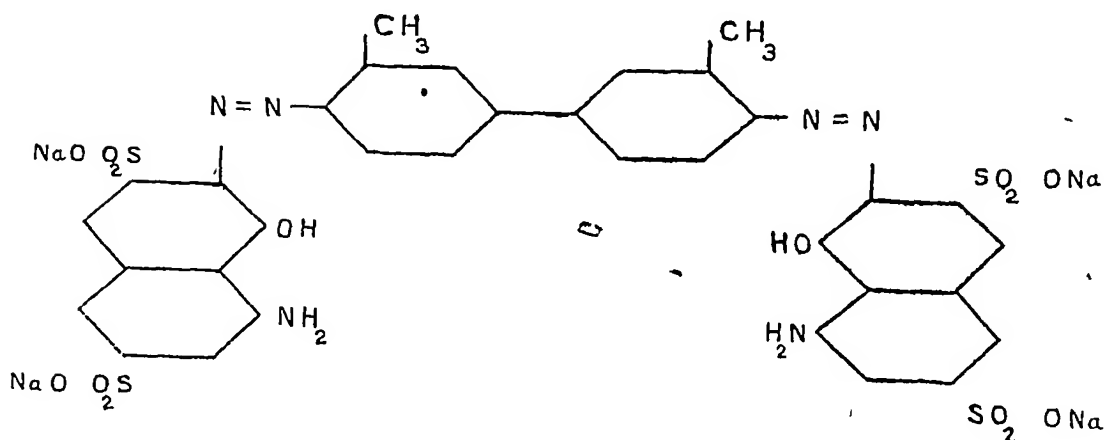
- 1 The entrance of mercury in the nucleus of phenol considerably enhances its bactericidal value
- 2 The bactericidal value of the chloromercuri group is greater than that of the acetoxymmercuri group
- 3 The para-chloromercuri compound is more active than the ortho compound

## II Compounds derived from dyestuffs

The compounds of this series were obtained by mercuriation of two benzidine dyes, Trypan Blue and Trypan Red, and one triphenylmethane dye, Acid Fuchsin

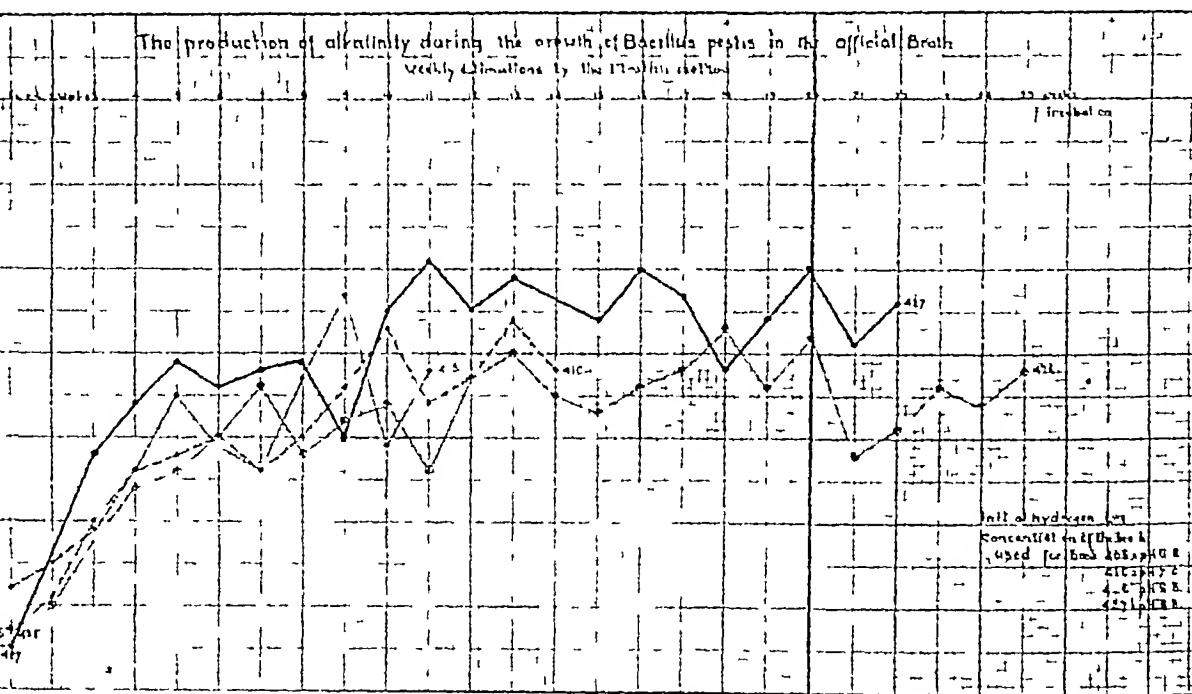


Benzidine



Trypan Blue

carried out experiments on four different brews, sown at different intervals, and incubated for periods varying from 85 to 176 days. The initial hydrogen-ion concentration of the four broths used for cultivation of plague bacilli was at pH 6.8 in three of them and at pH 7.0 in the other. For estimating the amount of growth of the plague bacilli and the degree of alkalinity produced in these media, we employed the following technique. After the preparation and adjustment of the hydrogen-ion concentration of broth in bulk, it was distributed into flasks with due aseptic precautions and inoculated with plague culture in the way employed here for the manufacture of the prophylactic. At the end of each week of incubation, the growth was tested for its purity, sterilised at 60°C for 15 minutes and tested for its sterility. No carbolic acid was added to the finished vaccine. First the opacity of the vaccine was estimated with the aid



of Brown's standard opacity tubes, and then it was filtered through filter paper several times until a clear filtrate was obtained. The hydrogen-ion concentration was determined by the colorimetric method using 5 c.c. of the filtrate and 0.5 c.c. of a 0.01 per cent solution of phenol-red as the indicator, then these 5 c.c. of the filtrate were titrated with N/50 hydrochloric acid to bring the hydrogen-ion concentration of the filtrate to pH 7.0 (neutral point). We had these estimations repeated by Messrs. Malandkar and Gokhale of the Biochemical Unit of this Laboratory, to whom we take this opportunity of tendering our thanks.

We have summarised the results, based on at least three titrations with each vaccine, carried out within a few days of each other, in the form of a graph.

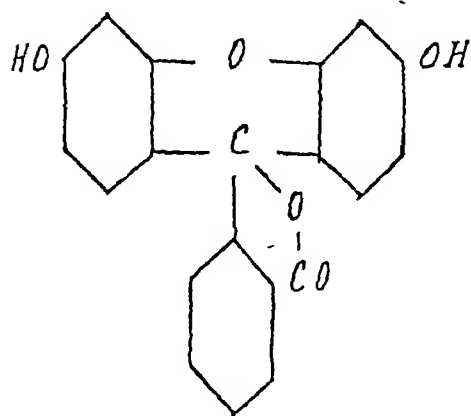
It was found that one molecule of Trypan Blue or Trypan Red could combine with as many as eight molecules of mercuric acetate, and that Acid Fuchsin combined with three molecules of the salt

The products were all soluble in water, and did not precipitate mercuric sulphide when treated with either sulphuretted hydrogen or yellow ammonium sulphide. But after destruction of the organic matter with sulphuric acid and potassium permanganate, and removal of the manganese dioxide by filtration, mercuric sulphide was precipitated on addition of sulphuretted hydrogen. From the weight of the precipitate the percentage of mercury in the compound was determined.

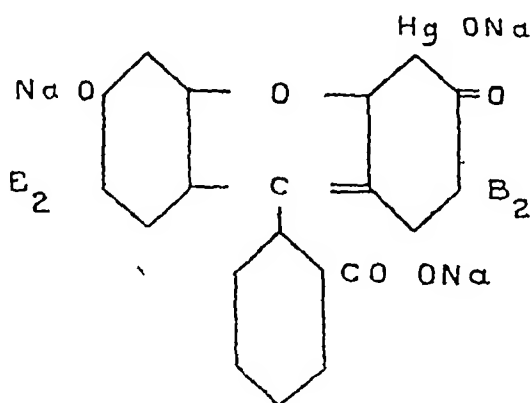
These substances are, therefore, acetoxymercuri derivatives in which the mercury is linked to the nucleus through one valency or to an acetoxy group through the other.

When warmed on the water bath the solutions of the penta-, hexa-, hepta-, and octoacetoxymercuri derivatives gradually throw down a coagulum which can be separated by centrifuging. Treatment with sodium hydroxide and alcohol showed that the coagulum is not a homogeneous substance, but a mixture of free mercury and dyestuff in varying proportions.

The bactericidal power of those mercurated dyestuffs is shown in Table II together with that of mercurochrome, a hydroxymercuri derivative of dibromofluorescein.



Fluorescein



Mercurochrome-220-sol

It may be seen from Table II that in chemically stable mercurated dyestuffs the bactericidal power is proportional to the amount of mercury present, and that little or no change is brought about by modification in chemical constitution.

TABLE I—*contd*

Vaccine incubated for weeks	Germ number 405 titrated on			Germ number 416 titrated on			Germ number 426 titrated on			Germ number 427 titrated on		
	10-7-1924	20-8-1925	20-2-1927	28-8-1924	21-8-1925	20-2-1927	16-1-1925	22-8-1925	21-2-1927	20-1-1925	25-8-1925	27-2-1927
13				44	44	46	40	39	37	49	45	48
14				38	40	38	35	35	34			
15							33	35	33	44	455	46
16							36	37	34	50	48	49
17							38	38	36	47	45	48
18							43	44	44	38	41	43
19							36	38	36	44	44	45
20							42	42	40	50	47	50
21							28	28	28	41	40	42
22							31	31	33	46	44	45
23							36	36	38			
24							34	34	34			
25							38	37	39			

From these results it would appear that the amount of alkalinity produced in different vaccines does not suffer any appreciable change even though the vaccines have been stored for varying periods from 767 days to 955 days

#### EFFECT OF INCREASED ALKALINITY OF THE PLAGUE VACCINES ON THEIR TOXICITY AND POTENCY

##### 1 *Heated but not carbolised vaccines*

We have carried out experiments to estimate the toxicity and potency of the vaccines which have not been carbolised and which have been incubated for varying periods of time, the technique employed is identical to that described in our paper on 'Notes on the potency of Haffkine's Plague Prophylactic' (4)

To determine with a greater degree of accuracy the effect of increased alkalinity on the toxicity and potency of the vaccine, we have here omitted all deaths occurring among the rats during the seven days following the immunisation with vaccine, where post-mortem examination failed to show

In general the bactericidal power is proportional to the amount of mercury present, and but little change is brought about by modification in chemical constitution

Experiments to ascertain the effect of certain of these mercurials will be carried out on plague infected animals in the near future

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TABLE III

*Vaccines incubated for 35 days and longer (35—74 days)*

Alkalinity of the vaccine in terms of the amount in ccs of N/50 hydrochloric acid required to bring 5 cc. of the filtrate of the vaccine to pH 7.0	Number of experiments	Number of rats used	'Toxic' deaths	Percentage toxicity	Survivors	'Plague' deaths	Percentage immunity
0.0—1.0							
1.2—2.0							
2.2—3.0	2	120	5	4.1	101	74	26.7
3.2—4.0	10	839	23	2.7	694	513	26.1
4.2—5.0 and over	34	1,559	64	4.1	1,306	879	32.7

TABLE IV

*Vaccines incubated for shorter periods (23—34 days)*

Alkalinity of the vaccine in terms of the amount in ccs of N/50 hydrochloric acid required to bring 5 cc. of the filtrate of the vaccine to pH 7.0	Number of experiments	Number of rats used	'Toxic' deaths	Percentage toxicity	Survivors	'Plague' deaths	Percentage immunity
0.0—1.0							
1.2—2.0							
2.2—3.0	8	300	8	2.6	250	171	31.6
3.2—4.0	19	630	18	2.8	505	342	32.3
4.2—5.0 and over							

From a study of these two tables it would appear that neither the toxicity nor the potency of the vaccines is appreciably affected by the variations in the amount of alkalinity in the finished vaccines.

## SUMMARY

1 During the growth of plague bacilli in broth, the medium becomes alkaline, this alkalinity of the medium steadily increases in amount from week to week reaching a maximum point between the fifth and the eleventh week of incubation, this is followed by a series of slight rises and falls in the amount of alkalinity throughout the period of incubation extending to 176 days.

PRODUCTION OF ALKALINITY BY *B. PESTIS* IN BROTH AND  
THE EFFECT OF THIS ALKALINITY ON THE  
TOXICITY AND POTENCY OF THE  
PROPHYLACTIC

BY

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[Received for publication, April 5, 1927 ]

HAFFKINE(1) was the first to notice that plague bacilli cultivated in broth ceased to multiply after an incubation period of five to six weeks, he found that this broth, in which the organisms had grown, would not feed any further growth of plague bacilli and was no longer a cultivating medium for this organism, he, therefore, concluded that this liquid medium is exhausted of its nutritious elements

Mayr(2) noted that if the broth was faintly acid at the time of inoculation with plague bacilli, it became alkaline when growth began to show itself, he found that this alkalinity was partly due to the volatile alkali, ammonia, he further noticed that this alkalinity of the medium increased *pari passu* with the growth of the bacilli, until it interfered with the further growth of the microbes

Bannerman(3) also found that plague bacilli grown in nutrient broth produced alkalinity of the medium, which alkalinity increased regularly from week to week reaching a maximum in six to eight weeks, and that this increased alkalinity caused the stoppage of further growth, although the plague bacilli may remain alive in such an alkaline medium for at least eighteen months Further, the neutralisation of such an alkaline medium resulted in the gradual increase once again of the amount of alkalinity due to the growth of plague bacilli, and, therefore, the nutrient elements in the culture medium were not exhausted, the stoppage of growth was really due to the amount of alkalinity reached by the medium Lastly, he considered that the alkaline body in the medium is probably not the plague toxin

PRODUCTION OF ALKALINITY IN BROTH BY THE *Bacillus pestis*

Bannerman's observations were based on experiments carried on a single brew of the prophylactic and only extended over a period of six weeks We have





From the graph it will be seen (a) that there is a gradual and steady increase in the amount of alkali produced from week to week reaching a maximum point between the fifth and the eleventh week, (b) and that this is followed by a series of small rises and falls throughout the period of incubation extending to 176 days

Agar cultures, made to test the purity of the cultures, yield a good growth of plague bacilli even though the broth culture has been incubated for 176 days

#### EFFECT OF STORAGE ON THE ALKALINITY OF PLAGUE VACCINES

With a view to determining the effect of storage on the alkalinity of plague vaccines, we have, at intervals of several months, estimated by the titration method the amount of alkalinity in vaccines, which have been heated, but not carbolised, and which have been stored at room temperature for varying periods of time

The results are summarised in the following table

TABLE I

Vaccine incubated for weeks	Germ number 405 titrated on			Germ number 416 titrated on			Germ number 426 titrated on			Germ number 427 titrated on		
	10-7-1924	20-8-1925	20-2-1927	28-8-1924	21-8-1925	20-2-1927	16-1-1925	22-8-1925	21-2-1927	20-1-1925	25-8-1925	27-2-1927
	cc.	cc	cc.	cc	cc	cc.	cc	cc	cc.	cc	cc	cc.
1	07	08		12	135	12	07	07	07	05	06	06
2	11	11		15	16		10	09	105			
3	20	20	20	19	22	19	18	17	16	28	28	26
4	26	26	25	26	26	26	24	23	24	34	35	34
5	35	34	34	28	28	28	26	28	24	39	37	39
6	29	27	28	30	33	31	30	28	30	36	355	38
7	26	27	28	26	27	26	36	34	37	38	375	38
8	37	37		30	33	31	28	30	27	39	38	38
9	47	43		36	39	36	32	31	33	30	32	33
10	29	29	31	43	42	40	34	34	33	45	47	44
11	38	38		34	35	33	26	25	25	51	475	50
12				37	39	36	36	34	33	45	46	

quinamine are known and the following table gives their mode of preparation and their physical properties —

*Isomerides and derivatives of quinamine*

Name and Formula	Formation	Properties
Quinamidine, $C_{10}H_{10}O_2N_2$	Action of Tartaric acid solution on quinamine at $130^\circ$	Crystals m p $93^\circ$ , [ ] D $+4.5^\circ$ in alcohol
Quinamicine, $C_{10}H_{10}O_2N_2$	By evaporating quinamine with sulphuric acid in alcohol	Crystals m p $109^\circ$ , [ ] D $+38.1^\circ$
Protoquinamicine, $C_{10}H_{10}O_2N_2$	By heating quinamine sulphate at $120^\circ$	Amorphous
Apoquinamine, $C_{10}H_{12}ON_2$	By heating quinamine or quinamicine with hydrochloric acid	Crystallises in leaflets or prisms m p $114^\circ$ , laevorotatory

Solutions of quinamine yield a yellow amorphous precipitate but no green colour when tested with chlorine or bromine water and ammonia. The solid alkaloid when moistened with strong nitric acid gives a yellow colouration.

A perusal of the literature shows that neither the action of quinamine itself nor its isomerides or derivatives have been investigated. We are very grateful to Mr G. E. Shaw, B.Sc., F.R.C.S., Quinologist to the Government of Bengal, for kindly supplying us with sufficient quantities of this alkaloid to enable us to study its pharmacological action.

• PHARMACOLOGICAL ACTION

*Action on undifferentiated protoplasm* — The bactericidal action of quinamine salts is less powerful than some of the other cinchona derivatives. Haemolytic streptococci are killed by a 0.5 per cent solution in  $2\frac{1}{2}$  minutes, but weaker solutions have no effect. In broth cultures *B. typhosus* is not killed by 0.1 per cent solution even in 10 minutes.

The lethal effect of quinamine on *Paramecium caudatum* was studied on the organisms cultured in hay medium. The results are tabulated below —

Dilutions of Quinamine	Time taken to kill	
	pH 7.82	pH 7.18
1 in 2000	4 minutes	6 minutes
1 in 3000	8 minutes	9 minutes
1 in 4000	18 minutes	20 minutes
1 in 5000	19 minutes	22 minutes
1 in 6000	One or two alive after 30 minutes	Just a few alive but sluggish after 30 minutes -

two or more of the signs which the Plague Research Commission had considered as signs of toxic deaths in rats(5) In estimating the potency of the vaccine we have also followed the Commission(6), by omitting from our figures those deaths which had occurred in the rats on the day of infection and the day following where post-mortem examination failed to show any of the naked eye appearances of plague and where microscopical examination of smears made from internal organs, such as the spleen and liver, failed to reveal the presence of plague bacilli

These results are here summarised in a tabular form

TABLE II

Alkalinity of the vaccine in terms of the amount in c.cs of N/50 hydrochloric acid required to bring 5 c.c of the filtrate of the vaccine to pH 7.0	Number of experiments	Number of rats used	'Toxic' deaths	Percentage toxicity	Survivors	'Plague' deaths	Percentage immunity
0.0-1.0	5	80	3	3.75	60	39	35.0
1.2-2.0	5	90	5	5.5	63	40	36.5
2.2-3.0	18	330	14	4.2	254	131	48.4
3.2-4.0	20	385	11	3.0	318	208	34.5
4.2-5.0 and over	10	215	2	0.9	192	121	37.0

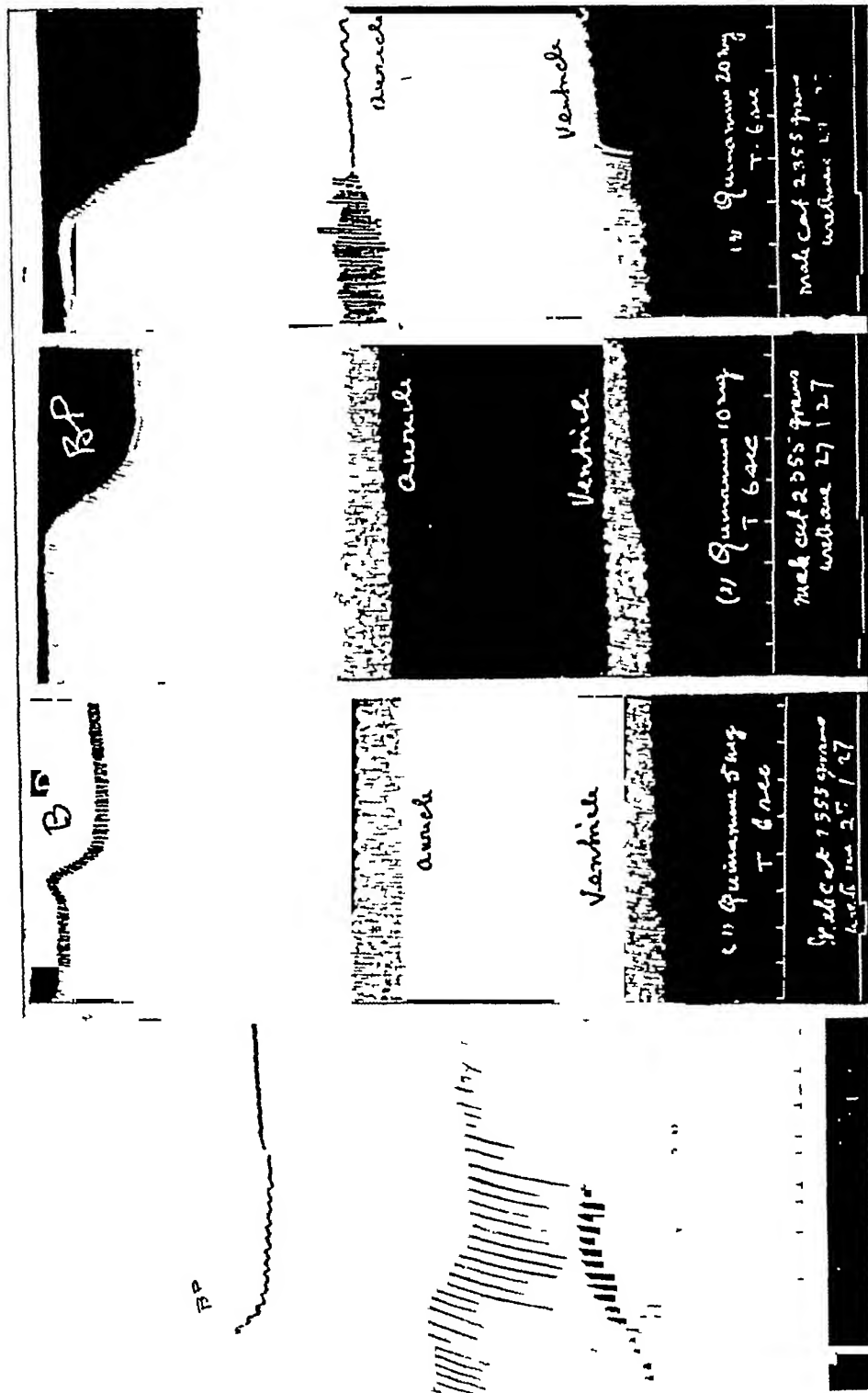
From the above table it would appear that there is no correlation between the amount of alkalinity present in the vaccine and its influence either on the toxicity or on the potency of the vaccines, in the doses that we have employed

## 2 Heated and carbolised vaccines

For the past fourteen months we have been regularly estimating the potency of the plague prophylactic brewed at this Institute for public use, we have estimated the amount of alkali produced in these brews by the titration method already referred to. The vaccines fall under two groups, namely, those incubated for 35 days and longer, and those incubated for shorter periods varying from 23 to 34 days to cope with the increased demand made on the vaccine. Both these groups have been carbolised so as to contain 0.5 cc of the antiseptic, after their sterilisation by heat. We have estimated their toxicity and potency in relation to their alkalinity.

The results of these experiments are here summarised

GRAPH II



2 Even though the broth culture of plague bacilli is incubated for a period of 176 days, the organisms are still viable and a good growth is obtained on agar slopes when subcultured

3 The amount of alkalinity present in the vaccine has apparently no influence either on its toxicity or on its potency as estimated in rats

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(35 mgm total) both the auricles and ventricles stop in diastole but can be temporarily revived with 1|20 c.c. of adrenalin (Graph II (d)). Quinine, quinidine, cinchonine and cinchonidine in 5-mgm doses sometimes produce a momentary stimulation of the auricular or ventricular beats when recorded by this method, but this is never noticed after quinamine. The slowing effect appears to be more marked with this alkaloid. It will, therefore, be interesting to see the action of this alkaloid in such conditions as tachycardia and auricular fibrillation.

*Respiration*—Quinamine administered intravenously to experimental animals under anaesthesia produces a well marked slowing and a slight decrease in the amplitude of the respiratory movements (Graph I (a) and (b)), there also appears to be some relaxation of the bronchi. Both these effects are not altered by section of the vagi.

#### GENITO-URINARY SYSTEM

*Kidney volume*—There is invariably a contraction of the volume of the kidneys corresponding more or less to the fall in the blood pressure. This, however, soon passes off.

*The Uterus*—The action of quinamine on the uterus was studied on the isolated uterus in the uterine bath and also in intact animals. The method adopted for determining the action of the alkaloid on the uterus *in situ* was a modification of the technique adopted by Barbour (1915). The animal was anaesthetised with urethane and the abdomen opened by a median incision about 3½ inches long starting at the level of the symphysis pubes. Two fingers were introduced into the abdominal cavity, the uterus was identified and one of its horns gently raised from its bed, great care being taken to injure the blood supply as little as possible. This horn including the ovary was carefully freed from its attachments and lifted into a vertical position by passing a thread through the broad ligament near its ovarian end. This thread was passed over a system of pulleys and was attached to the lever writing on the kymograph. The vertical portion of the horn was enclosed in a glass cylinder which was filled with normal saline at a temperature of 37.5°C. Owing to high atmospheric temperature the saline once put in the cylinder retains its temperature for a long time and it is not necessary to add warm fluid to keep the temperature at that point. Within about 10 to 20 minutes the uterus recovered from the shock and began to show its usual automatic movements.

*On Virgin Uterus*—The isolated virgin guinea-pig's uterus is markedly stimulated and the alkaloid in dilutions of 1 in 200,000 produces a well marked and persistent tonic contraction of this organ. Even 1 in 500,000 dilutions produce a slight but persistent tonic contraction of the organ (Graph III (a), (b) and (c)). The virgin uterus of the cat *in situ* shows tonic contractions when quinamine is given intravenously in doses of 3 to 5 mgms. If the uterus is relaxed with adrenaline and an injection of a few milligrammes of the alkaloid is given not only is the normal tone regained but tonic contraction is produced (Graph I (c)).

*On Pregnant Uterus*—The tone as well as the rhythmic movements of pregnant cat's uterus *in situ* are increased by 2 mgm given intravenously, while 4 to 5 mgm produce a well marked tonic contraction of the uterus (Graphs I (c) and III (d)).

# THE PHARMACOLOGICAL ACTION OF QUINAMINE.

BY

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QUINAMINE was first discovered by Hesse in the bark of *Cinchona succirubra* and has since been found in small quantities in the barks of *C. officinalis*, *C. rosulenta* and several varieties of *C. calisaya* particularly *ledgeriana*. It can be easily separated from the bark by the following process described in Allen's Commercial Organic Analysis

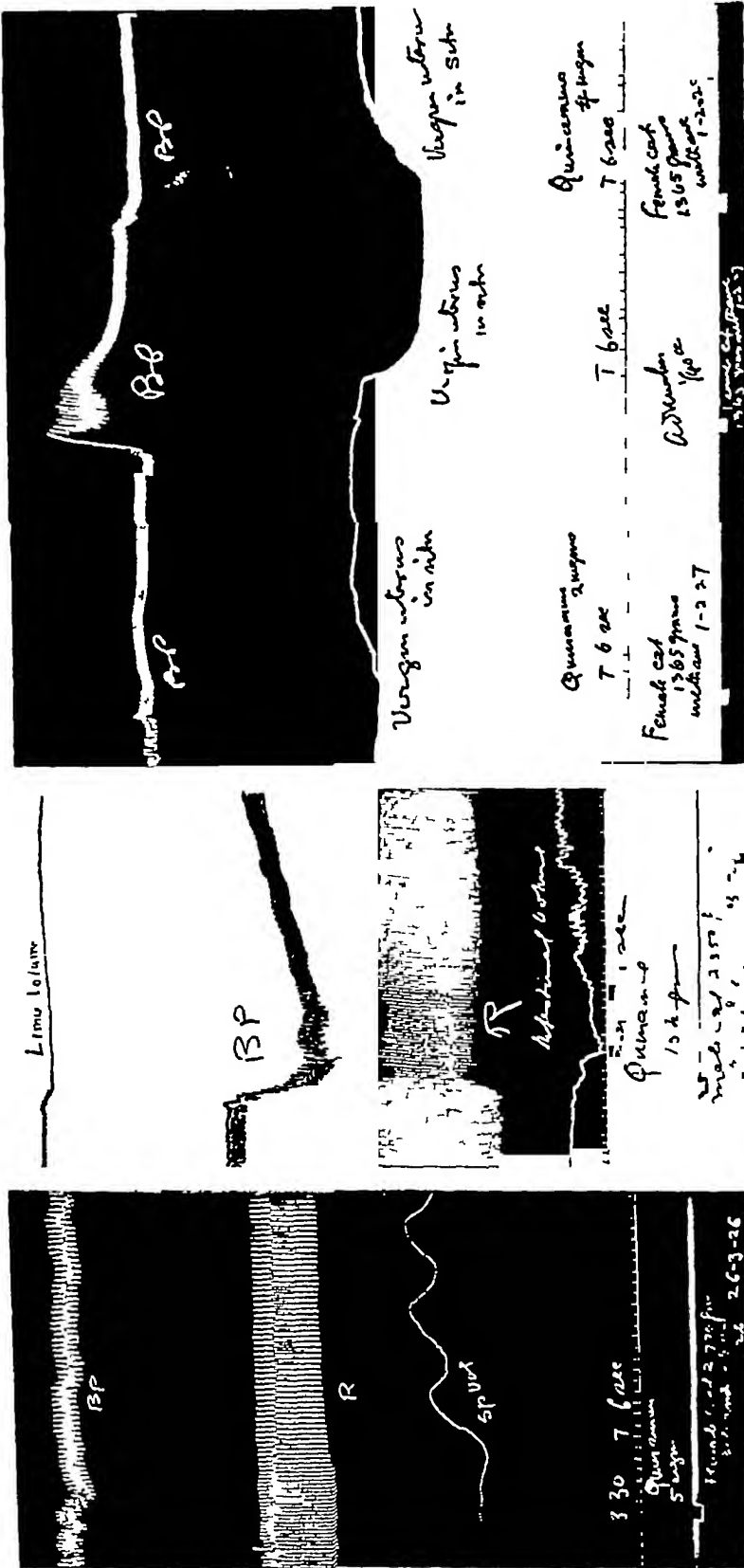
"The mother liquors from the crystals of quinine sulphate are precipitated with Rochelle salt, the filtrate treated with ammonia and the precipitate washed with ether. The ethereal washings are treated with acetic acid, the liquid neutralised, and while warm treated with potassium thiocyanate till on cooling, cinchonine can no longer be detected. Quinidine is then precipitated together with the colouring matter. The filtered liquid is treated with sodium hydroxide, and the resinous precipitate dissolved in a minimum of hot 80 per cent alcohol, from which quinamine crystallises on cooling."

Quinamine belongs to the dextro-rotatory group of cinchona alkaloids. It crystallises from dilute alcohol in long silky anhydrous needles which melt at  $172^{\circ}$  [  $\alpha$  ]  $D + 93.4^{\circ}$  in chloroform, and  $+ 104.5^{\circ}$  in 20 per cent alcoholic solution (97 per cent alcohol). It is nearly insoluble in cold water, but readily dissolves in boiling water. It is freely soluble in hot alcohol, in boiling ether, petroleum ether and benzene. It has no taste by itself but its solutions in acids are very bitter like quinine. The solution in excess of dilute sulphuric acid exhibits no fluorescence. Acid solutions of quinamine are easily decomposed with the formation of an amorphous alkaloid called quinamidine which is isomeric with quinamine. Quinamicine is also formed and under certain conditions apoquinamine,  $C_{19}H_{20}ON_2$ , is produced. A number of isomerides and derivatives of





GRAPH I



(a)

(b)

(c)

## SUMMARY AND CONCLUSIONS

(1) Quinamine occurs in appreciable quantities in cinchona bark produced in India (*succubia* and *ledgeriana*)

(2) The action of the alkaloid on the undifferentiated protoplasm such as bacteria or protozoa is not marked

(3) Quinamine has little effect on the digestive enzymes. It stimulates the peristaltic movements of the intestine and increases their tone. Intravenous injections cause an increase in the volume of the spleen and stimulate its contractions

(4) This alkaloid has a somewhat weaker action on the circulation than other alkaloids of the cinchona bark. The blood pressure falls but soon regains its normal level. Myocardiograph shows that the force and frequency of the heart are both decreased

(5) The respirations are slowed and there is slight but persistent dilatation of the bronchi

(6) The effect of quinamine on the uterus is very pronounced and even in dilutions of 1 in 500,000 it produces a well marked tonic contraction of the isolated uterus of cat and guinea-pig. Both the virgin and pregnant uteri *in situ* show well marked tonic contractions after injection of 2 to 5 milligrammes of the alkaloid. It is possible that quinamine is mostly responsible for the oxytocic properties attributed to quinine

(7) The anæsthetic action of quinamine on cornea and blocking of impulses in nerve trunk is almost as marked as that of cocaine

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| (4) DAVIES and SADTLER (1912) | Allen's Commercial Organic Analysis, Vol. VI   |
| (5) HENRY, T. A. (1924)       | Plant alkaloids  |

Acton (1921) found that at a pH of 7.25 the minimum lethal concentration for quinine, quinidine and cinchonidine is 1 in 10,000 and for cinchonine 1 in 3,000, whereas at a pH of 7.79 the minimum lethal concentrations for quinine is 1 in 50,000, for quinidine 1 in 100,000, for cinchonine 1 in 4,000 and for cinchonidine 1 in 50,000. Quinamine is therefore, much less toxic to *P. caudatum* than the other alkaloids of cinchona bark excepting cinchonine, and further the minimum lethal concentration does not differ materially within such a range of hydrogen-ion-concentration.

#### ALIMENTARY SYSTEM

We studied the action of the alkaloid on the various digestive ferments and found that it was very feebly active. On the digestion of starch by ptyaline as well as that of proteins by trypsin it has no appreciable effect. Dilutions of 1 in 50,000 and above have no action on peptic digestion but stronger solutions have a somewhat retarding effect.

In dilutions of 1 in 100,000 the tone of the isolated pieces of intestines is increased and the peristaltic movements are stimulated. The same phenomenon occurs when the alkaloid is injected intravenously in doses of 5 to 10 mgm in intact animals.

The volume of the spleen shows a definite rise and the rhythmic movements of this organ are markedly stimulated (Graph I (a)).

#### CIRCULATORY SYSTEM

Quinamine when given intravenously to experimental animals has only a slight depressing effect on the blood pressure. 5 mgm hardly produce any effect in a cat of average size, in fact sometimes a slight rise is noticed. 10 mgm produce a well marked fall, the pressure, however, soon regains its normal level (Graph I (a) and (b)). The fall of pressure is in no way affected when quinamine is administered after section of the vagi or after the vagal nerve endings are paralysed by atropine.

The fall of blood pressure is probably due to the direct depressing action of the alkaloid on the heart and the vaso-dilatation in the splanchnic region as is evidenced by the rise in the volume of the spleen. The pulmonary blood pressure falls slightly, corresponding to the fall in the systemic blood pressure.

The isolated mammalian heart when perfused with 1 in 60,000 dilutions of quinamine shows only a slight depression while dilutions of 1 in 120,000 have no appreciable effect. Stronger concentrations such as 1 in 12,000 produce a progressive slowing, weakness and irregularity of the beats but these soon pass off if the alkaloid is stopped, the heart regaining its normal rhythm and force. Graph II (a) shows the depression produced in the ventricle by 5 mgm of quinamine given intravenously, as recorded by Cushney's myocardiogram. It will be seen that the beats of both the auricle and ventricle are depressed and are visibly slowed after an injection of 5 mgm (Graph II (b)). After a further dose of 10 mgm in the same animal (15 mgm total) this effect becomes very much more marked, the ventricle is more depressed than the auricle and both the force and amplitude of the contractions is weakened (Graph II (c)). After a further injection of 20 mgm

the light of this research, porokeratosis is a localised ichthyosis with secondary atrophy of the epidermis in the centre of the lesion (morphœa). Hitherto the disease was considered to be resistant to the ordinary treatment by keratolytic agents such as salicylic acid, etc. Cases when treated in hospital responded at once to proper-thyroid medication.

*Distribution of the lesions*—The lesions are commonest on the hands especially on the dorsal aspect, and less commonly on the palmar aspect (Plate XXXIV). They may be present on the feet as well, with the same distribution as the hands. Plate XXXV shows a more extended distribution spreading up the arms to the side of the face and neck. Respighi and Ducery have described lesions inside the mouth in three out of four cases. Whilst Mibelli 1899 also found lesions in the mouth and on the glans penis in an extensive case of the disease.

*Course of the disease*—The lesions appear first as small horny elevations resembling a corn which slowly increases by a centrifugal spread, or as a thin callous spot. These areas gradually enlarge taking months or years before they reach any conspicuous size. The lesions may appear during childhood and slowly progress throughout life, new lesions developing from time to time (Case 6), but other cases do not appear until adult life or old age.

*Description of the lesions*—The edge is often irregular in outline but sharply defined against the outlying healthy skin, being raised hard or horny and darker in colour with the margin inverted or everted. Inside this wall is a seam or dyke which forms a narrow trough, the inner wall of the trough is bounded by a thin cord-like elevation. In the trough here and there are found small round areas of hyperkeratosis, which can be picked out leaving pits. The edge may be distinctly wall-like, perpendicular on its inner aspect, and on the outer side rapidly merging into the healthy skin. These edges may be fairly well rounded, but more often have a wavy irregular outline. The enclosed portion is always lower than the general skin surface and has an atrophic depigmented appearance, with slight scaliness or a wrinkled surface owing to the normal lines of the skin being slightly widened, and a smooth surface owing to the disappearance of the lanugo hairs. On the mucous membranes the lesions appear as opalescent, rounded or irregular patches which are limited by a white raised border and surrounded by a slight zone of hyperæmia. There may be no subjective symptoms but as a rule the lesions are irritable, and the sweat and sebaceous secretions are in abeyance owing to obstruction of their ducts. The affection does not affect the general health, but as it occurs frequently on the hands, the patients come for advice owing to the unsightliness of the lesions.

*Morbid anatomy*—Sections were made from Cases 1 and 2. In the former, the whole lesion was excised and, in the latter, a section was made through the edge and centre of the growth. The photograph (Plate XXXVI) shows the structure of the microscopical section. At the edge of the lesion the vessels can be seen larger and dilated, the horny layer is heaped up into a peak-like projection which shelves on the outer side into the healthy skin, and on the inner side the wall is straight cut and ends in a dyke below the surface level. The horny cells are imperfectly cornified, and the pigment is increased in the basal layer on the outer side of the wall. As one proceeds inwards, the vessels in the corium are surrounded by

# GRAPH III

Virgin guinea  
pig's uterus  
(isolated)



(a)

Quinine  
24-6-26  
2

(b)

Quinine  
24-6-26  
2

(c)

Pregnant uterus  
(cat) in situ

T. 6 sec

Quinine 4 mgm  
Femur cat 1000g  
weight 28-1-27

(d)

there was a definite history of inheritance, in the others this point was not carefully asked for from these patients. As the result of my work on diseases like asthma, etc., 1924-1925, I showed that the causation of many of these diseases was bound up with the endocrine defence mechanism, and the degree of their function was frequently inherited from the parent. As the result of my studies on skin diseases such as ichthyosis, etc., where there is a family history very frequently present, I came to the conclusion that many of these so-called hereditary diseases were bound up in a similar way with the endocrine function and were inherited from father to son, just as colouration, texture of hair, etc., is inherited and closely related to the function of the various endocrine glands.

*Causation*—Tommasoli was the first to show that he considered porokeratosis to be an abnormal form of hyperkeratosis of the skin, which was not in any way a different disease. We have been able to persuade two of these patients to come into the hospital and in both of them the basal metabolic rate was very low indicating that there was some deficiency in the action of the thyroid gland. This finding has been an almost invariable rule in all our cases of ichthyosis, follicularis, etc. Moreover, with proper thyroid medication, these cases have been cured so rapidly and completely as to suggest that there was some direct association between the thyroid functions and the occurrence of these hypertrophies.

On studying the deficient function of the thyroid such as is seen in myxœdema, the skin is not hypertrophied, nor do we see conditions such as ichthyosis, etc. I would suggest that there is probably some other factor which controls the growth of the skin in such areas, and that the thyroid function is indirectly associated with this growth principle so that when there is hypofunction of the thyroid we see the action of the growth factor, and when there is normal or high function of the thyroid this principle is kept in check. I do not believe that the association of the thyroid and these hypertrophies is a direct one, because sometimes the basal metabolic rate is not markedly diminished, and sometimes, though rarely, the thyroid treatment fails. The hereditary effect on the thyroid gland can thus be explained, and so the disease would tend to be a family one.

*Treatment*—Hitherto, porokeratosis has been regarded as an incurable disease, and the treatment recommended has generally been a keratolytic ointment, curettage, or the use of caustics. Lately, I have been using thyroid medication in all these cases, and the results have been very striking in our indoor patients, and of very little use in the outdoor. In the indoor patients the metabolic rate is first taken and, according to the degree of deficiency, the dose of thyroid is regulated, if it is diminished between 15 and 20 per cent, we generally give 2 grams of the dried thyroid gland twice a day and the pulse is recorded every four hours. The dose of the thyroid is increased or diminished so as to keep the pulse rate between 90 and 100. In cases where the metabolic rate is diminished more than 20 per cent we generally give 3 grams twice a day, and the pulse is recorded four-hourly. Within a few days a very marked improvement is noted and usually a cure occurs within two weeks. In the cases treated in the out-patient department, where large doses of thyroid cannot be given, this improvement is not seen to the same extent and it appears that the thyroid treatment is not effective unless the patient is at rest.

*On Multiparous Uterus*—The reaction of the multiparous uterus to quinamine is very similar to that of the virgin uterus, both the tone and the automatic movements are markedly increased

Acton (1922) showed that with 1 in 40,000 dilutions quinine throws a pregnant uterus into a high state of tonus while 1 in 100,000 only increases the intermittent uterine contractions. Weaker dilutions such as 1 in 180,000 have no effect whatever. Quinamine, however, causes marked tonic contraction of the uterus in such high dilutions as 1 in 500,000, and in stronger concentrations the effect becomes more and more marked. It will be seen therefore that quinamine in concentrations in which it is likely to circulate in the blood after absorption, has a well marked action on the contraction of the uterus and is likely to produce abortion. It is possible that the idea prevalent about quinine producing abortion in pregnant women was due to the presence of this alkaloid as an impurity with quinine, as Acton's work has shown that none of the other alkaloids have any marked stimulating effect on the uterine contractions in the concentration in which they circulate in the blood. It is a well known fact that most of the commercial preparations of quinine on the market are not pure and contain appreciable quantities of other cinchona alkaloids. The oxytocic action of quinamine should also be borne in mind when prescribing cinchona febrifuge, which contains all the alkaloids of cinchona bark including quinamine, to pregnant women.

#### NERVOUS SYSTEM

Quinamine in dilutions of 1 in 750 produces partial anæsthesia of the rabbit's cornea in about 5 minutes, with stronger solutions complete anæsthesia is produced, as shown in the following table—

Dilutions of quinamine	ANÆSTHESIA	
	Produced in	Lasting for
1 in 500	5 minutes	About 7 minutes
1 in 200	4 minutes	About 10 minutes
1 in 100	4 minutes	About 15 minutes
1 in 50	3 minutes	About 30 minutes
1 in 25	2 minutes	About 35 minutes

1 in 50 or 2 per cent solutions of cocaine and 1 in 25 or 4 per cent solutions of quinamine produce anæsthesia of the rabbit's cornea in 2 minutes and the anæsthesia lasts for 21 minutes in the former and 30 minutes in the latter case.

A 4 per cent solution stops the conduction of impulses through the nerve of a sciatic-gastrocnemius preparation of a frog in about 12 minutes. With cocaine of the same strength the same result is obtained in about  $9\frac{1}{2}$  minutes.



Plate XXXIV —Illustrates the lesions in Case 7. The more recent lesions appeared as flat cornified areas which later became atrophic and depigmented with a raised wall round the atrophic area.

# POROKERATOSIS ITS CAUSATION AND TREATMENT

BY

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*Synonyms*—Hyperkeratosis eccentrica (Respighi), keratoderma eccentrica, hyperkeratosis figurata centrifuga atrophicans, Fr porokeratoses

*Introduction*—In 1893, this rare skin condition was first described by Mibelli as a hitherto unrecognised type of hyperkeratosis, occurring on the extremities as small eccentrically shaped patches. The name porokeratosis was given to it by Mibelli, for he considered it to be essentially a hyperkeratosis occurring round the sweat pores. In the same year and independently Respighi 1893 also described the disease. Tommasoli 1894 was the first to question the individuality of this hyperkeratosis, as he believed it was only an unusual example of other hyperkeratosis, such as ichthyosis, linear nævi, etc. Mibelli in 1895 was convinced from the clinical features, histological findings and the course and behaviour of the disease that it was strikingly different from any known skin disease. Macleod 1920 states that about 60 cases were on record and it had been observed in England, France, Germany and America. My excuse for writing about this condition is that during the last few years I have seen no less than eight cases of porokeratosis. Moreover, I agree with Tommasoli that it is merely a type of hyperkeratosis, which spreads in an irregular centrifugal manner. With the increase in size atrophic changes take place in the centre of the lesion. I will further show that the condition is partly correlated with hypofunction of the thyroid gland. In Calcutta I see every year a number of these conditions of hypertrophy of the various layers of the skin, and have classified them under four main headings.

(i) Hyperkeratosis of the horny layer, ichthyosis, (ii) hyperkeratosis of the hair follicles, follicularis, (iii) hypertrophy of the corium, scleroderma, and (iv) hypertrophy of the deeper layer of the corium and atrophy of the surface layer, morphœa. They are all partially related to hypofunction of the thyroid gland. This work which is as yet unpublished is in preparation, and I hope to bring together some 30 odd so-called skin diseases under these four headings. In



endothelial cells and the melanoblasts are not seen so that the pigment in the basal layer is much diminished, the horny layer is lost and the surface is covered by flattened epithelial cells. With the increase in the blood supply at the edge there is an increase in pigment production and in the heaping up of the horny layer, but with the pressure caused by the hypertrophy of the endothelial cells, the blood supply is diminished so that both the pigment and the production of horny cells is diminished, leaving the surface layer white and atrophic in appearance. Sections were stained in various ways for spirochetes and other organisms, but none were found.

*Description of the Cases*—Case 1 Hindu male, aged 20, with a single lesion situated on the inner side of the nipple. The lesion was removed by excision.

Case 2 Mohammedan male, 28 years of age, history of the disease for two years, no family history recorded. The legs were in an ichthyotic condition. A section was made from the lesion and the patient was treated with thyroid and salicylic ointment. He attended for two weeks and was not seen again.

Case 3 Hindu male, 22 years of age, with a single lesion on the back of the left hand. He had noticed it for one year. The case was treated with carbon-dioxide snow and healed leaving a scar.

Case 4 Mohammedan male, 30 years of age, lesions on the hands and feet, chloasma on the face. Was treated with thyroid and salicylic ointment and was cured.

Case 5 Mohammedan male, aged 32, duration of the disease six months, treated with thyroid and salicylic ointment in the outdoor and was almost cured. His father also suffered from the disease.

Case 6 Hindu male, 35 years of age, duration of the disease 22 years. Lesions situated on the side of the face, neck, arms and legs (*see* Plate XXXV). He gave a definite history of the disease occurring in his family and in several of his brothers and sisters.

Case 7 Mohammedan male, aged 16 years of age, was admitted into hospital on the 26th of January, 1927. The lesions were distributed mainly on the hands and feet (*see* Plate XXXIV). His brother was also suffering from the disease. The disease appeared early in life and there were exacerbations on and off during the winter months. The basal metabolic rate was taken and was found to be 35 per cent below normal. He was placed on thyroid treatment on the 1st of February and by the 8th, the lesions had completely healed. No local treatment was given.

Case 8 Mohammedan male, aged 20. The disease appeared early in life, and the single lesion was similar to that of his brother which lasted for a year. The basal metabolic rate was taken and found to be 20 per cent below normal. He was placed on thyroid treatment on the 29th of January, 1927, and on the 8th of February, the lesion disappeared and the thyroid treatment was discontinued. The basal metabolic rate in both of these brothers was taken on the 10th February, and was found to be respectively 5 per cent in Case 7 and 4 per cent in Case 8 above normal.

*Ætiology*—The disease was first shown by Gilchrist to be associated with some factor which was inherited from the parent. In the Cases of 5, 6, 7 and 8



# CONCLUSIONS

(1) Porokeratosis, although a distinct clinical entity, is merely a form of hyperkeratosis

(2) Clinically it is characterised by the occurrence of raised corn-like lesions, which as they enlarge form an irregular raised wall, which surrounds an atrophic depressed centre

(3) Histologically, it is characterised by an increased vascularity of the papillary vessels which causes acanthosis and hyperkeratosis. With the continued vascularity the endothelial cells and fibroblasts multiply in the corium, constrict the vessels by pressure and atrophy sets in, causing the depressed depigmented wrinkled centre

(4) The association of a familial tendency and the lowered basal metabolic rate suggests that there is a partial correlation with hypofunction of the thyroid gland

(5) The correlation is not a complete one as these lesions are not found in other conditions of hypofunction of the thyroid such as myxœdema and cretinism

(6) The lesions can be cured in two to three weeks by proper thyroid medication

(7) Porokeratosis is therefore a localised keratosis with central atrophy (morphœa) and falls into the large group of so-called skin diseases, xeroderma, scleroderma, follicularis and morphœa, which are all partially associated with hypofunction of the thyroid

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Plate XXXVI —Microphotograph of a section through the lesion showing (1) the raised cornified wall, (2) the dyke below the wall, (3) the hypertrophied papillæ, (4) the floor thinning out by pressure atrophy of the papillary vessels









skin It is therefore possible to get these angiomata occurring at three different levels of the skin in either a diffuse or nodular form

*Definition*—Sarcoids are acquired venous angiomata that are produced by proliferation of the lining of these vessels, which in turn is caused by the irritation of bacteria or some circulating toxin The clinical types that will be described, therefore, depend on the position at which the venous plexuses are involved in this hypertrophy

*Clinical types*—(1) The commonest type that is seen are the small venous angiomata which are situated superficially, and were first described many years ago by Morgan at the Middlesex Hospital when he noticed them in association with cancer of the bowel These spots are spoken of as the *tâche de Morgan* They are seen as small angiomatous areas which are flat in appearance and rise above the surface level of the skin They are bright red in colour owing to the vessels being filled with arterial blood, as they are derived from the finest venules formed from the arteriole tufts of the papille These spots are very frequently seen on the trunk, in persons who are over 40, and are generally associated with some septic focus in the alimentary canal, it may be the teeth, throat, stomach or gut They often appear suddenly and grow to their maximum size about 3 millimetres in a few days time They may persist as such or undergo spontaneous cure It appears that these spots are derived from the fine plexus of venules situated immediately under the basement membrane

(2) The type described by Boeck consists of two clinical varieties—

(a) *The nodular type* (Plate XXXVII) This is the commonest clinical variety that I have seen During the last year (1926) I saw over 20 cases, all of whom had suffered from epidemic dropsy The nodules vary in size from a millet seed to a small hazel-nut, and raise up the epidermis Occasionally these nodules grow very rapidly, forming large tumours the size of a small walnut The rapid growth is largely due to an œdema which is produced in the subcutaneous tissue by diffusion of serum from the venules and gives the histological structure of the tumour an appearance like a nasal polypus (Plate XLI, fig 3) The lesions occur chiefly on the trunk, less numerous on the hands and sometimes involves the mucocutaneous junctions of the nose and anus

(b) *The plaque type* The plaque type occurs as dark red plaques which extend much lower down in the cutis than appear at first sight to the eye On palpation the thickness of the plaque can easily be felt when it is moved on the subcutaneous tissue In the erect position, when of large size, they swell up above the skin surface and subside when lying down at rest The commonest site of the lesions are on the backs of the hands and face Plate XXXIX shows a large single plaque of the thigh In the centre of these plaques there is often a distinct slightly pale coloured orange area, which is hard to the touch and feels more solid than the vascular red area In both these types spontaneous recovery is often observed, so that one has to be careful in appraising the value of any remedy used for treatment

(3) The subcutaneous sarcoid of Darier and Roussy In this type the lesions are much deeper and situated in the region of the subcutaneous tissue,

Plate XXXV—Illustrating Case 6 The lesions are more extensive and extend to the side of the face

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PLATE XXXVI





# ACQUIRED ANGIOMATA OR SARCOIDS

BY

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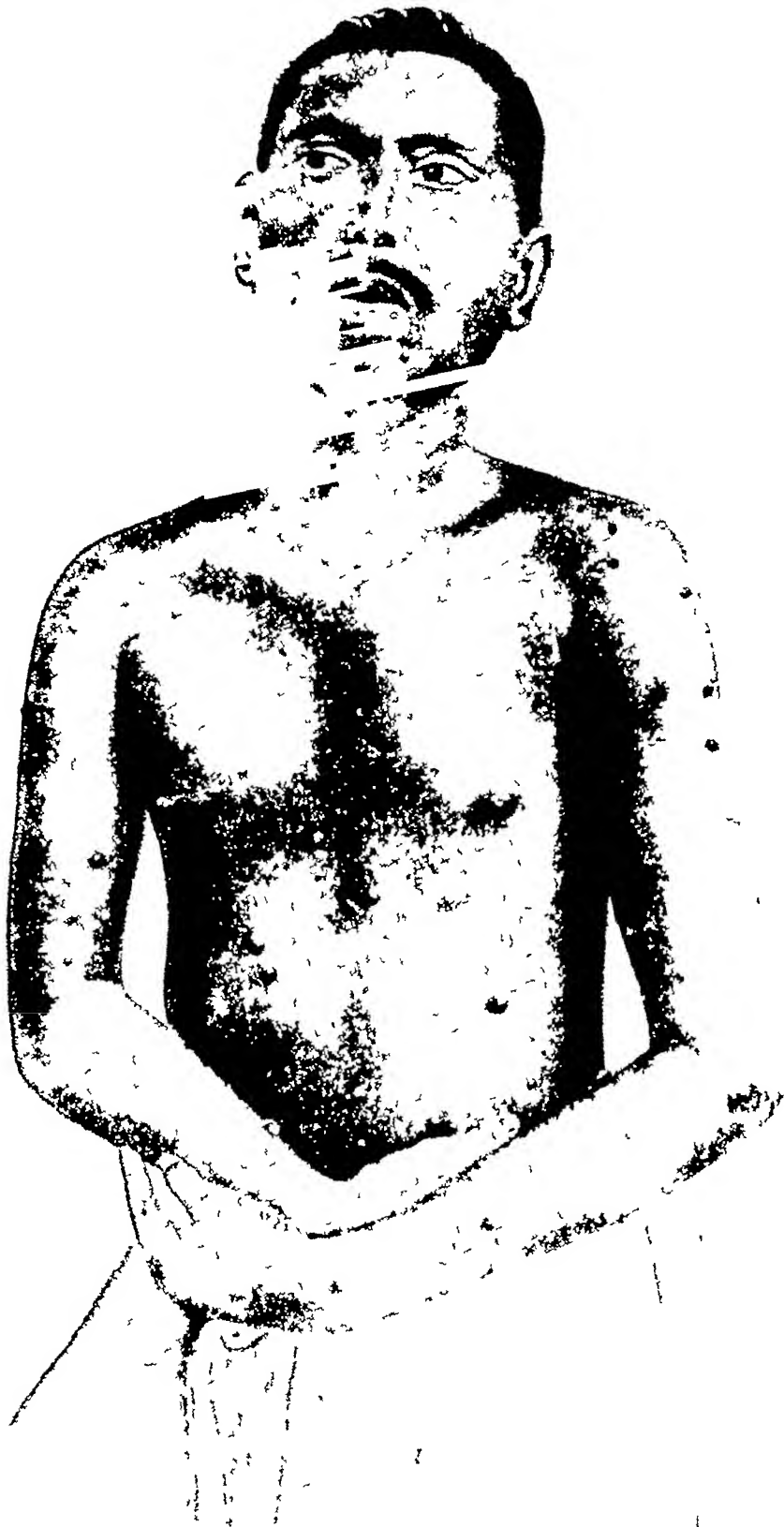
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*Introduction*—The term sarcoid is an extremely unfortunate name for these lesions, as they bear no relationship or resemblance to the sarcomata. The name has caused a great deal of confusion, because it had been previously used by Kaposi for a group of diseases which were either sarcomata or resembled these neoplasms very closely. These diseases were mycosis fungoides, leukemic tumours of the skin, Kaposi's hæmorrhagic sarcoma, and sarcoma cutis. Boeck (1899) was the first to remove them from these malignant neoplasms. He used the term sarcoid for a group of lesions which were characterised by the formation of nodules, or diffuse infiltration of the skin or subcutaneous tissue, and which were innocent in nature. The lesions were usually followed by atrophic scarring without ulceration, and thus resembled Crocker's nodular lupus erythematosus. Darier and Roussy (1904) described a type of lesions which they called subcutaneous sarcoids. Still later (1910), Darrier divided the sarcoids into four types of cases: (1) multiple benign sarcoid (Boeck), (2) subcutaneous sarcoid (Darier & Roussy), (3) a nodular variety resembling erythema induratum, and (4) Spiegler-Fendt type resembling leukemia cutis. Most authorities are of the opinion that the Spiegler-Fendt type resembles histologically a lymphomata, and should not be classified under this head. There is a difference of opinion about the third type, which is considered by many to be a variety of erythema induratum (Bazin).

During my experience at Calcutta I have seen at least 20 cases of the nodular type, 6 cases of the subcutaneous sarcoid type of Darier Roussy, and two cases of the erythema induratum type. From a study of the histology and mode of production, I am convinced that these skin lesions are acquired angiomata, which are caused by the action of bacteria or poisons coming from the gut and setting up hypertrophy of the endothelial lining of the venules at different levels of the







Shows the nodular type of acquired angiomas occurring in a patient who had suffered from Beriberi





The nodular type rapidly growing owing to œdema occurring in the connective tissue of the growth

if deeper down we get Boeck's nodular type and when the subpapillary venous plexus is involved the Darier Roussy's type, finally we get an erythema induratum type in the subcutis. The hypertrophy of the endothelium may be isolated to certain venous areas giving rise to the flat or discoid type, or it may be more diffuse and spread centrifugally giving rise to the plaque type. If situated in the subcutaneous tissues, the spread of the growth may also take place centripetally giving rise to the nodular type.

On examining sections of the skin in epidemic dropsy, we saw that there was an enormous dilatation of all the skin vessels in the different areas (Plate XLI, fig 2). The venules in the subcutaneous fatty tissue are seen to be large and dilated, at first sight looking as if hæmorrhages had taken place in this area. In the vessels under the epidermis there is a distinct proliferation of endothelial cells round these vessels, which indicates irritation by some agent.

Sections made from the tache de Morgan shows that the hypertrophy of the vessels occurs immediately under the basement membrane, so that the colour of the blood is almost arterial in character, which gives these angiomatas a bright red colour. There is no doubt these spots are due to a hypertrophy of the blood vessels of the papillary venous plexus which in turn is produced as a result of stimulation of the angioblasts by certain septic conditions of the gut, whether due to bacteria, emboli or to the action of toxins is at present unknown.

Sections on the nodular type of sarcoids (Plate XLII fig 4), show that there is flattening of the papillæ of the epidermis, œdema in the subpapillary tissue and the formation of a large number of new blood vessels extending into the corium. These vessels have very thin walls and resemble veins rather than arterioles. The dilated venules are seen chiefly in the region of the subpapillary plexus, and the smaller venules in the central and more solid part of the growth form solid masses of cells which penetrate deep down into the corium. Fig 4, Plate XL, shows a sarcoid which is fungating and has become papillomatous. Here the epidermis is very thin and the papillæ are flattened out over the tumour. The main mass of the tumour consists of numerous newly formed vessels separated from each other by myxomatous looking tissue, which is really due to the white fibrils being separated from each other by œdema. Towards the base of the pedicle of this growth the fibrous tissue is thickened in two directions, fibres running parallel to the base, and bands of fibres that follow the main blood vessels into the tumour mass. Here and there were large dilated venous spaces which contain, mainly, coagulated serum with a few blood corpuscles at the periphery of the space. There is no evidence of any inflammatory changes or the suggestion of malignancy.

In the diffuse type of sarcoid Darier Roussy, the growth has a distinct nævoid character with numerous blood vessels extending from the basement membrane right down into the corium (Plate XLII fig 5). The avascular yellow looking areas were found to be made up of these venules that had collapsed and formed solid plugs of endothelial cells. In many places the venule wall consists of more than a single row of endothelial cells. The close relationship between these angiomata and inflammatory processes occurring in the body was discovered first by Morgan, but is now being extended by me to the whole of these sarcoids.



The plaque type of acquired angiomas. Note the denser and yellowish red area in the centre of the lesion.





The erythema induratum type of acquired angiomata—duration of the disease, 12 years





they do not as a rule extend to the skin surface When this occurs the diagnosis is only possible by section cutting

(4) The sarcoids of the extremity resembling erythema induratum Plate XL shows these lesions on the right thigh and leg of a girl of about 18 There was no surface ulceration, the history was of 10 years duration and the nodules approached the skin surface, colouring it a deep purple colour

*Ætiology*—Morgan was the first observer to point out that the spots that had been named after him and called *tâche dé Morgan*, occurred only in elderly people and generally preceded cancer of the alimentary canal In 1926, we saw a number of cases of the nodular type of Boeck, which occurred secondarily to an attack of epidemic dropsy In patients suffering from epidemic dropsy, we found that a large number of them were passing micro-organisms in their urine, and that these bacteria were derived from the alimentary canal, because they belonged either to the coliform group or to the gut streptococci When the patients recovered from the dropsy, these angiomas tended to disappear Therefore, it appears that in epidemic dropsy the toxins or micro-organisms can, in exceptional cases, irritate these vessels and set up hypertrophy with the result that new vessels are formed giving rise to angiomas Dr José N Rodríguez informed me that he has seen similar cases in Manila, Philippine Islands, that occurred in patients suffering from beri-beri, at that time he was not familiar with the clinical aspects of these lesions

In the plaque type occurring on the hands and face, we were able to find a septic focus in the teeth These were dealt with and the patients treated with vaccine therapy and apparently cured by treatment In the case depicted in Plate XXXIX we were at first unable to find the septic focus, but on culture of the urine we obtained a hæmolytic diphtheroid organism from which a vaccine was prepared, and after a course of six injections the lesions cicatrized Previous to this the patient had had a course of streptococcal vaccine made from his teeth without any benefit The nodular type seen were all secondary to epidemic dropsy Most of the cases were seen in the men who came to the hospital for treatment for epidemic dropsy There were four cases amongst the women who were admitted into the hospital, in whom the skin condition was diagnosed during their stay in the hospital The majority of these cases were between the ages of 18 and 45

*Morbid anatomy and pathology*—It is first necessary to describe how the vessels are situated in the skin, as there is considerable confusion in their exact distribution in the skin according to the description in many text-books on dermatology The venous return of the skin can be considered to consist of (*see* Plate XLI, fig 1)—(1) A fine plexus which is situated directly under the basement membrane of the papillæ and which has fine regular meshes This plexus is followed by two closely situated plexuses with a large mesh We may consider these as the papillary venous plexus (2) A larger plexus which corresponds to the subpapillary arterial plexus From this plexus venules go to the larger veins situated in the subcutaneous tissue under the corium, so that it is possible to get hypertrophy of the endothelial cells at these different levels We can, therefore, see that if hypertrophy occurs in the superficial plexus we get a *tâche dé Morgan*,

plexus, (iii) the Darier Roussy type deeper down in the cutis, and (iv) the erythema induratum type

(3) These angiomata are acquired as the result of irritation of vessels in the different skin layers by some septic condition of the gut, either due to bacteria or toxins

(4) As the result of the action of the toxin, an increased permeation of these vessel walls occur, giving rise to rapid growth in the size of the nodule, or the pressure of the growth may collapse the vessels in the centre of the lesion

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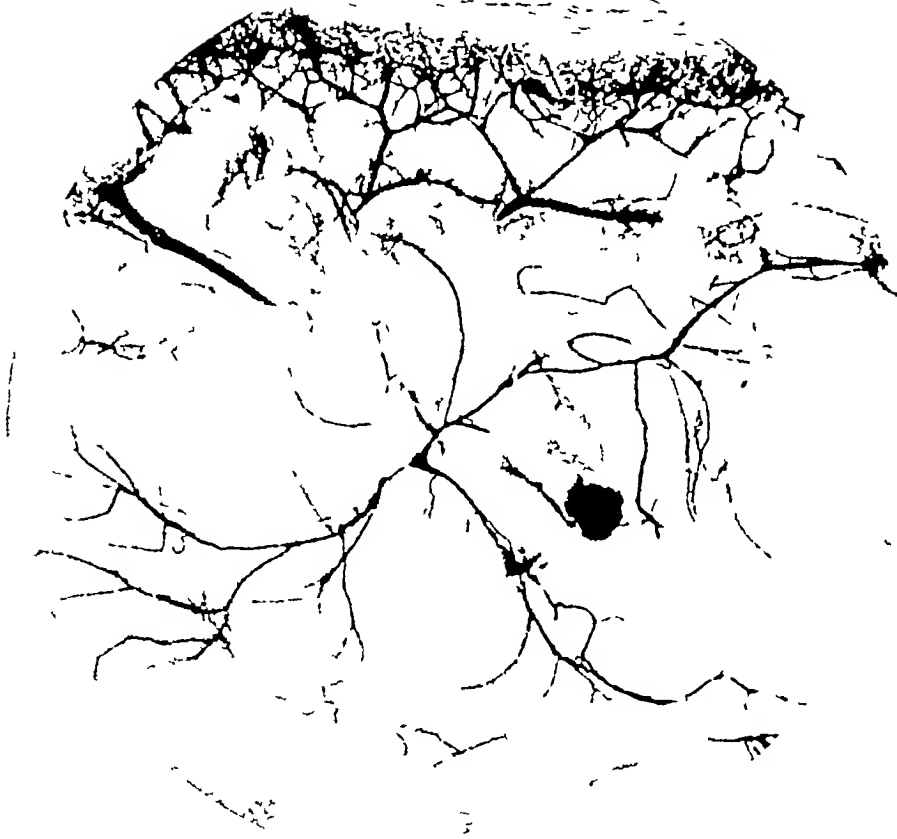


FIG 1—Section of a foetal skin injected with Indian ink showing papillary plexus, sub-papillary plexus, vessels in the subcutaneous tissue and deep plexus 1 inch objective



FIG 2—Section of subcutaneous tissue from epidemic dropsy showing fat cells and dilated blood spaces 1/12 objective.



FIG 3—Fungating sarcoid showing flatten blood vessels and oedematous tissue 1/3

was found, however, that what we gained in extent of the work was more than outweighed by the loss in intensity and while mere survey for the vibrio might be carried on by this arrangement, special study of the organism and its vectors was not possible. So after working in these two areas from November 1925 to May 1926, we set up a laboratory at a place near Calcutta in June last and concentrated our investigation on the villages of Thana Baruipur and its neighbourhood in the district of 24-Parganas.

3 *Methods*.—The first part of our work may be regarded as of a preliminary nature. During this period, we had to test, adapt and work out methods, both for collection of materials from villages for examination and also for isolating the bacilli. We began with faeces tubes for bringing stools to our laboratories. But the time taken, often over 12 hours, proved too much for the faeces to remain fresh, and, consequently, for the vibrio to survive in the collections. For such cases of delay in transport, Nicholls (1917) recommends calcium carbonate. Panganibon and Shobl (1918) found the *Vibrio cholerae* surviving in sea water from 106 to 120 days. They made further investigation on the preserving action of solution of sodium chloride and showed that in concentration of 0.5 to 5 per cent, the vibrios were preserved at least for 5 weeks, and that, even when these organisms were in dilution of 1 to 100 million parts of a loopful of emulsion in 10 c.c., they lived up to 6 weeks in 1 per cent solution of the salt. After trying for some days the antacids, sodium carbonate and calcium carbonate, we have therefore used 1 per cent salt solution for the purpose. Dr. Tomb and Capt. Maitra of the Asansol Mining Settlement also give this method of collection and conveyance in their article in the *Indian Medical Gazette* of February 1926.

For isolation we have been using the Dunham's peptone salt water for enrichment followed by sowing in ordinary nutrient agar (pH 7.6). Opinions vary as to the optimum content of the salt in the peptone water for culturing the vibrio, Sierakowski holds it to be 0.5 per cent, Beauveries (1916) suggests 3 per cent as the strength which gives the thickest pellicle, while Kabelik and Freudmann (1923) find that the optimum salt concentration varies generally with the pH value of the culture medium, and that 3 per cent is more useful than 0.5 per cent. But as the vibrio, though growing vigorously in the 3 per cent solution, ages quickly and shows involution forms and loss of motility at such concentrations we keep to 0.5 per cent, the pH of the medium being 7.6. We find the nutrient agar with 0.5 per cent of sodium taurocholate the best solid medium for isolation of the vibrio, it grows in the medium rapidly and the colonies are too characteristic to be missed. We do not see any necessity for using special selective media or the bile salt lactose agar of MacConkey. We do not follow the usual process of inoculating the liquid culture into melted agar tubes at 40°C and then spreading the agar on Petri dishes to set (since the vibrio is too delicate for exposure to such a temperature) but use Petri dishes spread over with the agar and dried free of the water of condensation making repeated streaks with one and the same charge on the surface of the agar, thus getting colonies which are quite discrete.

4 *Vibrios in human stools*.—(1) In Khulna and Mymensingh. The result of our work of the first seven months, i.e., from November 1925 to May 1926, was published as Research on Cholera in Rural Bengal, 1925-26, by the Public Health

PLATE XLII

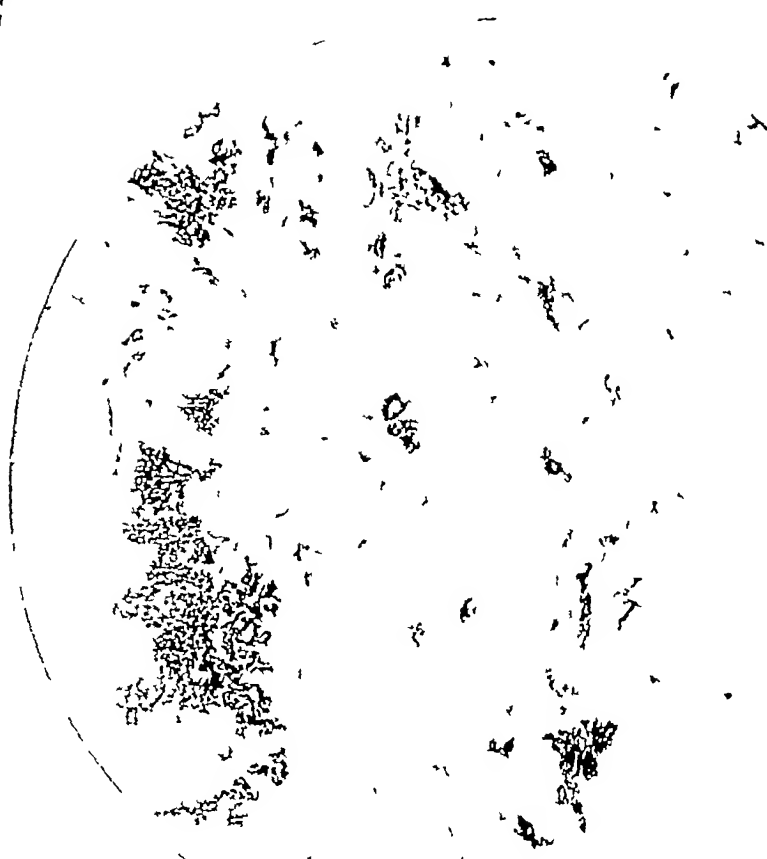


FIG 4—Section of a nodular sarcoid showing epidermis and blood spaces extending into the corium 1/3 objective

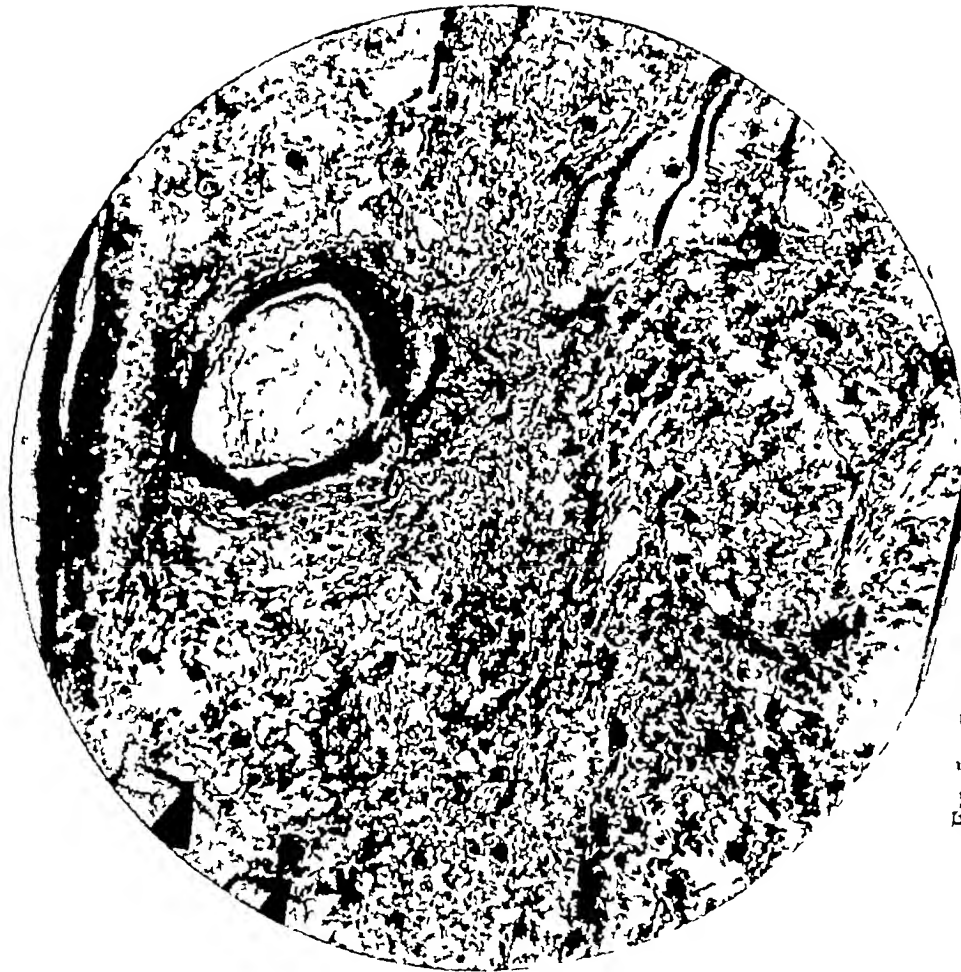


FIG 5—Section of the erythema induratum type showing flattened epidermis, hair follicle undergoing hypertrophy of the horny layer (follicularis) and dilated venous spaces

showed him all the details of his methods of collection and isolation. Our mode of transport of the stools was the same as his. His process of isolation [by standing collecting tubes for some hours at room temperature, transferring 6 loopfuls from the emulsion to 250 c.c. of 1 per cent salt solution in sterilised tank water in bowls, to which 1 c.c. of 1 per cent peptone water has been added and testing it daily till the vibrio is found and then inseminating it into ordinary peptone water for intensive growth] did not appeal to us, as it entailed so much loss of time without any decidedly commensurate advantage in our actual practice. So we have kept on with our direct transference from the emulsion of faeces in the salt solution of the collection tube to the Dunham peptone water as soon as the tube reaches our Laboratory. Our results, too, though they appeared to us to be low, compared favourably with that obtained at the Mining Settlement, our figures for vibrio carriers being 0.6 and 0.2 per cent of our two populations as against 0.1 per cent of that of the Mining Settlement (Tomb and Maitra, 1926).

(2) In 24-Parganas. On page 23 of my report on the work of 1925-26 referred to above, I recorded my suspicion that our low result was due to the smallness of the quantity we took as sample and proposed mass cultivation, i.e., making an emulsion of the whole stool, or, at any rate, of a large part of it. In practice, carrying such a large quantity of the faeces of each person from the villages to the laboratory was found unworkable. So we have to be content with increasing the quantity examined to 4 of 5 grams collected in test tubes 6 ins.  $\times$  1 in. containing 30 c.c. of 1 per cent salt solution. The result has been satisfactory, the number of vibrio carriers found in the villages of Thana Baruipur and the neighbourhood from June to December 1926 was 231 out of 1,377 villagers examined, i.e., 12.4 per cent. The endemicity of cholera in these areas according to the registered mortality from the disease compares with the percentages of the vibrio carriers found, as follows —

Area	MORTALITY PER MILLE PER ANNUM FROM CHOLERA IN THE QUINQUENNIAL 1921-25			Percentage of vibrio carriers
	Maximum	Minimum	Average	
Khulna	14	0.4	0.9	0.6
Mymensingh	0.7	3.8	1.7	0.2
24-Parganas	0.8	3.3	1.7	12.4

The figure for 24-Parganas is 20.7 times that of Khulna and 62 times that of Mymensingh. Obviously such a large excess of vibrio carriers is only apparent, being due to the improved method enabling us to find out the organisms in cases where we would be missing them by working with the smaller quantities we previously used.

5 *Significance of the vibrios* — (1) Agglutinating vibrio. Our standard cholera anti-serum is the immune serum obtained by the Calcutta Medical College

It will therefore be seen that these angiomas consist of vessels in different degrees of potency, which gives rise to the difference in their colour, the different clinical types being due to the amount of the vascular area involved and to their anatomical situation. These angiomas are reflections on the skin surface of some underlying inflammatory process in the body. They are therefore quite distinct from the erythema induratum (Bazin) which is a tuberculide and the Spiegler-Fendt type which is a leukaemia cutis.

*Diagnosis*—The diagnosis is easy provided that one is familiar with the look of these lesions, and the possibility that acquired angiomas arise as the result of some septic process in the body. The commonest mistake is to diagnose the plaque type as leprosy, especially if the lesions are situated on the face and hands. In Europeans, the lesions are of a very much darker colour than in leprosy, and in the centre there are often yellowish areas. The mistake in diagnosis is readily corrected as soon as an attempt is made to cut the edge of the lesions to examine for leprosy bacillus, as the snip is very painful and bleeds profusely.

The rapidly growing fungating type of nodular sarcoids are sometimes mistaken for sarcomata owing to their rapid growth, but microscopical sections will show the structure to be like a nasal polypus with no evidence of any malignant change.

*Prognosis*—The prognosis is good, as many of these cases undergo spontaneous cure. The treatment by vaccines after finding the septic focus also appears to help in the cure. When these cases are rebellious to treatment it generally means that the actual septic focus producing the lesions has not been found, and hence keeps up the disease.

*Treatment*—When the lesions are small they are best treated by an application of CO<sub>2</sub> snow applied for about 10 seconds, which causes the rapid disappearance of the angioma. In the diffuse type where the area is large, constriction of the vessels is first brought about by the use of evaporating lotions such as lotio calamine.

As regards the general treatment, the first thing to do is to find where the septic focus exists in the body. Usually in those cases which involve the face and hands the focus is found in the mouth, either in the teeth, as peridental abscess, or in the tonsils. When the lesions are generalised such as the nodular form seen in epidemic dropsy, the focus is in the alimentary canal. In the case of the large plaque-like lesion on the thigh, the organisms were found in the urine. If possible, the septic focus should be removed by the extraction of the teeth, or the removal of the tonsils. The causative organism is isolated, a vaccine prepared and graduated doses given over a course of 6 to 8 injections. So far all my cases have responded well to vaccine-therapy. In the nodular type associated with epidemic dropsy when the gut returns to a normal condition, the sarcoids usually disappear spontaneously within a few weeks time.

#### CONCLUSIONS

- (1) Sarcoids are angiomatous growths, which are innocent in nature.
- (2) Four types are described (i) the tâche de Morgan in the papillary venous plexus, (ii) Boeck's type which is nodular or diffuse in the subpapillary



It will be seen from the table below that in all these stools, from the villages as well as from the Calcutta hospital, no agglutinating vibrio was found till November

Month	STOOLS OF CHOLERA PATIENTS			Agglutinating vibrios found in	Percentage
	Villages	Calcutta hospital	Total		
June	12		12	nil	
July	7		7	nil	
August		25	25	nil	
September		22	22	nil	
October		4	4	nil	
November	2	21	23	8	34.4
December	1	37	38	13	34.2
TOTAL	22	109	131	21	

Absence of agglutinating vibrios in the choleraic stools of the quiescent period was in consonance with the findings of other workers. The low percentage in the stools of November and December was due to our missing the early stage of the disease when taking the samples. The five instances in which the standard vibrio was found in the stools of the contacts and the controls were also all of November and December, viz —

Class	Month	Number examined	Agglutinating vibrios found in	Percentage
Persons with previous history of cholera	July-Oct	92	nil	
	Nov-Dec	13	nil	
Contacts	July-Oct	721	nil	
	Nov-Dec	137	2	1.5
Controls	Sept-Oct	107	nil	
	Nov-Dec	292	3	1.0

(2) Non-agglutinating vibrios.—The remaining 226 vibrios did not react to the standard immune serum. Morphologically, they were all unflagellate and all had the characteristic appearances of the *Vibrio cholerae*. Eight of them inoculated into rabbits showed no specific agglutinin. But the mere fact of their not responding to the specific agglutinin or of their lack in specific agglutinin cannot be regarded as excluding possible identity with the agglutinating standard vibrio. Pottevin has seen strains, agglutinating highly on isolation, frequently losing the power to do so, and non-agglutinating strains, on the other hand, taking on the power as suddenly. Minervin (1913) could select from agar plates, sown with the

# ON THE PREVALENCE OF *VIBRIO CHOLERÆ* IN SOME OF THE ENDEMIC AREAS OF BENGAL

BY

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*Introduction*—Though the *Vibrio cholerae* was discovered in 1883, our knowledge of its prevalence in communities (apart from patients and in direct connection with the disease) is still meagre. Researches that have been carried out have for the most part been concerned with the nature of the organism, its virulence and pathogenicity. However, as the result of this work, and I should mention specially the researches of Col Grieg (1913, 1915), we now know that the vibrio is not limited to patients and convalescents—it is harboured by those who have had the disease not only recently but over twenty years ago, as well as by healthy persons living in contact with cholera cases. We have also instances of healthy carriers, i.e., persons having no history of choleraic symptoms in themselves, spreading the disease, as in the outbreak in Oranie recorded by Sergeant and Negre (1912). The key to the source of epidemics and, for the matter of that, our ability to control the disease with certainty lies in discovering these healthy porters of infection. This side of the problem has attracted attention of late in India, and Capt Maitra is now working on it in the industrial population of the Mining Settlements of Asansol. Our own work applies to the village homes of the settled population of the province, to such villages as are not likely to have the endemic prevalence disturbed by convection of the disease from outside, as is the case with all industrial localities.

2 *Endemic areas under investigation*—At the start we took up two areas in different parts of the province, one in Central Bengal and the other in Northern Bengal. Each of these areas, or circles consisted of a number of villages within a reasonable distance and easy of access from the head-quarters, where we opened a laboratory. Our object in working simultaneously at two distant parts was to arrive at a fair idea of the distribution of the vibrio over the whole province. It

December, which means 3 non-agglutinating vibrio carriers in every 8 persons and 1 such carrier in every 4, respectively, during these epidemic months

Distribution of these organisms in persons differently related to the disease is anomalous. From June to August we examined stools of the patients, of persons with previous history of the disease, and of those who lived in the infected houses, it was from September that that we could examine also those who had no connection with the disease. The following table shows prevalence of the non-agglutinating vibrios among the different classes of persons examined from September to December

Class	Number examined	Number found with non-agglutinating vibrios	Percentage
Patients	87	33	37.9
Persons with previous history	55	10	18.2
Contacts	359	37	10.0
Controls	399	104	26.1

While the ratio of carriers decreased regularly from actual patients, through those with a previous history of cholera, to the contacts, it rose very high in the controls, being more than one in every four such persons, a very unlikely state if these organisms were all transformed cholera vibrios. Analysis of their prevalence in the persons with a previous history of cholera, shows that the time elapsing since the attack makes no difference in the ratio of carriers among these persons

Year of attack	Number examined	Number found with non-agglutinating vibrios
1926	70	10
1925	23	3
1924	1	.
1923	2	1
1922	6	
1921	2	2
Over 15 years	2	

(ii) The average case mortality in cholera in the villages is 50 per cent, the rate of mortality from the disease is about 2 per mille of the population per annum, the rate of attacks is, therefore, about 4 per mille. We have seen above that the non-agglutinating vibrios were recovered from 37.5 per cent of healthy persons

Department, Bengal The find of vibrios in stools, excluding those of ten acute cases of which six returned agglutinating vibrios, was as follows —

Circle	Number of persons whose stools were examined	NUMBER OF PERSONS IN WHOSE STOOLS VIBRIOS WERE FOUND		Percentage of vibrio carriers in the total examined
		Agglutinating with standard anti-serum	Not agglutinating with standard anti-serum	
Khulna	3,598	3	18	0.6
Mymensingh	2,176	3	5	0.2
TOTAL	5,774	6	23	0.5

Of the six vibrios agglutinating to the standard anti-serum, 3 were obtained from convalescents and 3 from persons who developed the disease 5 days after taking of the samples, the faeces from two of these latter were hard and from the third, soft, there was cholera at the time in both the two villages to which these persons belonged. As to the nature of the non-agglutinating vibrios, the following table showing their relative prevalence in persons having different relations to the disease will be interesting —

History	Number of persons whose stools were examined	NUMBER OF PERSONS IN WHOSE STOOLS VIBRIOS WERE FOUND		PERCENTAGE OF VIBRIO CARRIERS TO TOTAL OF THE CLASS	
		Agglutinating	Non-agglutinating	Agglutinating	Non-agglutinating
Patients	10	6		60	
Convalescents	17	3		17.7	
Persons with previous history of cholera	179		2		1.1
Contacts	1,335		6		0.4
Controls	4,243	3	15	0.07	0.3
TOTAL	5,784	12	23	0.2	0.4

Even if all the non-agglutinating vibrios were true cholera vibrios the number of carriers would have been only 0.6 per cent for the villages of Khulna and 0.2 per cent for those of Mymensingh, whereas only a fraction of non-agglutinating vibrios could presumably be true cholera, i.e., variants of agglutinating strains. So we were afraid our findings were below the actual mark. I therefore sent one of my assistants to the Asansol Laboratory and Capt. Maitra very kindly

As the population of Thana Barupur is 102,609, the chances, at the different carrier rates from different numbers of persons examined would be as in the following table —

Carrier rate per cent	Number of persons examined	CHANCE, PER CENT OF		Remark on number examined as regards inference
		Missing carrier	Hitting carrier	
0.4	300	30	70	Not sufficient
0.4	1,000	2	98	Fair
0.4	2,000	0.3	99.7	Sufficient
1	300	5	95	Fair
0.1	1,000	40	60	Too small
0.1	2,000	13	87	Fair

Still as the true cholera vibrio is known in the laboratory to lose its reaction to the standard immune serum, the occurrence of the same phenomenon under natural condition is not an unlikely contingency. Hence, we have taken 69 non-agglutinating strains including 21 from healthy persons and 19 from water for close study, our object being to find out means for detecting such among them as may be really transformed true cholera vibrio. We have been studying them serologically with a view to reduce them into classes. 17 of the vibrios from cholera patients, 13 from healthy persons and 5 from water have already fallen into 7 groups. The sera of each of these groups act only on the members of the respective groups at high titres, but have no action either on the standard vibrio or on the members of other groups. Only two strains were exceptions, they exhibited group agglutination, agglutinating with sera of two different groups at a comparatively low titre of 1/3000 to 1/1000. In this respect, therefore, the seven groups behaved as so many distinct species in the same sense as the different members of dysentery typhoid, Gartner groups are so many distinct species. The result of the serological investigation will be given in a subsequent paper. Morphologically, as already mentioned, they are all motile, unflagellate and of the characteristic shape. Detailed study of their size and shape, as well as revision of our research on the power of both agglutinating and non-agglutinating vibrios for carbohydrate fermentations, indol formation, hæmolysis, salt precipitation and change and longevity in water will be taken up after we have finished the serological work. It is only after we have been able to reduce these numerous vibrios into their species by these means, that investigation for the altered pathogens will be possible.

#### SUMMARY

With a view to finding out the distribution of cholera carriers, we examined, from November 1926 to May 1927, simultaneously, stools of 3,598 persons in villages of Central Bengal and of 2,176 persons in those of North Bengal. In spite of our improving the methods of collection and transport of the stools to the

primarily from Kasauli and since then prepared at the College and at our own laboratories by immunising rabbits with a vibrio reacting at extreme titre to the standard anti-serum. Such an agglutinable vibrio is pathogenic without question, as it also gives response to the sera of indubitable cholera patients. Out of the 1,377 persons of those villages examined from June to December, 6 showed this vibrio in their stools, the ratio being 0.4 per cent.

The prevalence of the agglutinating vibrios in persons according to their relation to the disease was as follows —

Class	Total examined	Number of persons showing agglutinating vibrios in their stool	Percentage
Patients	22	1	4.5
Persons with previous history of cholera	105	nil	.
Contacts	857	2	0.2
Controls	399	3	0.7
TOTAL	1,377	6	0.4

The two contacts showing agglutinating vibrios had cholera in their houses at the time of collection of their stools, so they might, after all, be acute carriers under the same category as the actual patients, being probably cases of infection not developing into the disease. But the carriers among the controls, i.e., persons who had no relation with the disease, were found at Kalyanpur, a village which, though a scene of recurrent outbreaks of cholera, had no case of the disease at that time. The details of the cases were as follows —

Month	PARTICULARS OF THE CARRIERS		Titre of agglutination	REMARKS
	Sex	Age		
November	Female child	7 years	1/2000	Examined up to the titre of 1/2000, pending finding of the limit at the final examination
"	Male	20 "	1/2000	
December	Female child	5 "	1/2000	

All these three cases were in different houses.

Seasonal variation — The only case among the 22 patients in whom agglutinating vibrios were found occurred in November. Besides the stools of these cholera cases from the villages, we also examined those of 109 from a Calcutta hospital



pure agglutinating vibrio, colonies with all degrees of reaction down to its complete absence, Stamm (1914) by passages in water, each lasting as a rule through 5 days, found that genuine strains agglutinating with 1 20000 specific serum failed to react later at 1 50 but produced on injection into rabbits anti-sera acting not only on itself but also on the original strain, Puntoni (1913), by growth in water, as well as in earth and water mixture, removed not only the response to the specific agglutinin on several occasions, but also the specific agglutino-gen, the immune sera produced in animals by injecting these degenerates acting on themselves only and not on the unaltered strain Crendropoulo, too, as the result of examination of faeces of 34,461 passengers from infected countries and of other persons, has been led to formulate his dictum (Defressin and Cazeneuve, 1914) that 'every carrier of vibrios who comes from an infected or suspected country ought to be treated as a suspect irrespective of whether the vibrio isolated agglutinates with the specific serum or not and conversely every carrier of vibrios from an uncontaminated locality must be looked upon as harmless likewise irrespective of whether the vibrio isolated agglutinates or not'

(a) Are then all our 226 non-agglutinating vibrios from human stools altered standard *Vibrio cholerae*? Such would be the case according to Dr Tomb and Capt Maitra

(i) Statistically, the data are too small for basing conclusions of any accuracy Still, in their prevalence, these vibrios display an apparent regular variation through the seasons, which is compared with the mortality from the disease in the table below

*Non-agglutinating vibrios in stools of healthy persons, i.e., persons with previous history of cholera, contacts and controls*

Month	Persons examined	Non-agglutinating vibrios found in	Percentage	Number of deaths from cholera in Thana Barupur	Rainfall in inches
June	78	3	3.8	7	11.9
July	178	10	5.6	3	12.5
August	296	17	5.8	3	12.7
September	351	17	4.8	2	9.9
October	20			1	4.2
November	213	80	37.5	22	0.7
December	219	59	26.9	7	0.2
<b>TOTAL</b>	<b>1,355</b>	<b>186</b>	<b>13.7</b>	<b>45</b>	

Keeping between 4 to 6 per cent during the quiescence of the disease they jumped up to 37.5 per cent in November, coming down only to 26.9 per cent in



Strain of lymph	Number of calves vaccinated	Average yield in grammes per calf	Vaccination rate on calves	RESULTS ON CHILDREN			
				Number of children vaccinated	Case success rate	Total number of insertions	Number of insertions unsuccessful
Rabbit			Continuous lines				
Buffalo				34	100		Nil
Calf 1	201	23.1		140	100	480	0.41%
Calf 2	259	23.5		110	100	400	2.05%
Calf 3	88	22.5		50	100	200	3.0%
Calf 4	39	24.4		40	97.5	160	6.2%
Calf 5	15	20.5		20	100	80	3.8%
Calf 6	6	36.8		14	92.6	54	7.4%
Total	608	23.3	Mostly continuous lines, discrete vesicles in a few cases, rate never below 1/4	408		1,414	34

in November of the last year. If these organisms could cause cholera, over a third of the population would have been carriers of the disease in that month.

(iii) Serologically, not only did these vibrios not react to the standard anti-serum, but the sera made by injection of eight of these strains into rabbits did not act on the standard vibrio. A standard vibrio which has lost its specific agglutininogen may produce an anti-serum which, while acting on the degenerate vibrio, has no power on the original strain, but such anti-sera are always weak. The anti-sera produced by the vibrios under discussion were all of high potency excepting one, the titre limit of which, however, was still  $1/4000$ .

(b) If these non-agglutinating vibrios are not altered cholera vibrios, yet they may contain among them some which are pathogenic.

(i) Para-cholera vibrios—Greig found a non-agglutinating vibrio which agglutinated with the serum of the host, i.e., the cholera patient from whose stool it was recovered, the patient passing no typical *Vibrio cholerae* and his serum not acting on the latter vibrio. We also have obtained a vibrio from a patient which, while not agglutinating with the standard immune serum, was positive with the serum of the patient, which again had no action on the standard vibrio. We have reserved this vibrio for further study.

(ii) Altered cholera vibrio—Some of these vibrios may be the true *Vibrio cholerae* which have lost the capacity of agglutination with the standard serum.

It is not the absence of agglutinating vibrios in our samples taken during the quiescent period that leads us to this suspicion. The lack may be due simply to the paucity of data. We have already shown that among the 1,355 healthy persons examined from June to December, the number of those in whose stools we got the agglutinating vibrio was 5, i.e., about 0.4 per cent. In the month of December, too, the ratio of such persons was about 0.4 per cent, the agglutinating vibrio being found in the stool of only one out of the 219 healthy persons examined in this month. As December is an epidemic month, the carrier rate, i.e., the ratio of healthy persons harbouring the agglutinating vibrio could not be more than 0.4 per cent for the year, in all probability, it was less in the off season, i.e., in the months in which the disease was not active. We did not get any such carriers among the persons we examined from June to October. But the number of stools examined in the different months varied from 178 to 357 being even lower, viz., 78 in June and only 20 in October.

Now  $p$  being the population

$n$  being the number of carriers

$x$  being the number of persons examined

$$\text{Chance of missing the carriers would be} = \frac{p-n}{p}^{c_x}$$

$$\text{Chance of finding the carriers would be} = 1 - \frac{p-n}{p}^{c_x}$$



laboratory and of enrichment and isolation, we found the agglutinating vibrio only (excluding ten patients) in 3 convalescents and 3 contacts, and in 23 more we found non-agglutinating vibrios, the total for carriers of all vibrios being only 0.5 per cent

We later examined villages near Calcutta and increased the quantity of the stools taken for examination. From June to December we examined stools of 1,377 villagers. Healthy persons from whom we got the agglutinating vibrios were two contacts and three persons having no connection with the disease, the total rate being about 0.4 per cent. The number of persons carrying non-agglutinating vibrios were 13.7 per cent.

What is the significance of these data? If the agglutinating vibrios only were the cholera vibrios, 0.4 per cent of the people being carriers of the disease would sufficiently explain its endemicity. If so, how is one to account for its absence in the stools during the off season?

Could it be due to the number of persons examined, about 200 a month, being too small?—Not an unlikely explanation.

Could it be that the true vibrio loses its agglutinability and hence is classed with the non-agglutinating vibrios in these months? Seasonal variation of the latter seems to lend colour to the view, but it is not supported by their relative prevalence in persons with different relations to the disease.

To elucidate the relation of the non-agglutinating vibrios to the typical *Vibrio cholerae*, we have been studying 69 such strains including 19 from water. The result of the investigation will appear in subsequent papers.

In concluding this preliminary paper, I may mention that I am much indebted for the work carried out in the laboratory to skill, diligence and devoted application of my assistants Dr S. C. Chatterjee, M.B., and Dr S. K. Ghoshal, M.B., D.P.H.

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| PUNTONI (1913)                  | <i>Trop Dis Bull</i> , Vol 2, p 383  |
| DEFRESSINE and CAZENEUVE (1914) | <i>Trop Dis Bull</i> , Vol 3, p 478  |
| TOMB and MAITRA (1926)          | <i>Ind Med Gaz</i> , Vol 61, p 538   |

*Scheme of work*—It was proposed to make one collecting trip during the three pre-winter months, and again another during the three winter months, and a programme was drawn up accordingly

We visited one or more centres in a district and stayed in each for about a week and collected larvæ and adults from places in and around it. Railway journeys were the means of transport on most days but the motor buses and taxis at some places were used. Every morning the collector went to a different place. His visits were arranged by reference to the 1 in = mile G. I. survey maps which show excellently such features as bils. He was advised to catch from different types of breeding places in each locality—but special observation was to be directed towards the “dead or dying rivers,” “running streams,” “Bils,” “large clear water tanks” long railway cuttings, borrowpits for huts, etc

We visited 25 districts out of the 27 in Bengal—the Chittagong hill-tracts and Rajshahi being the two omitted, due to the close of the work in the 3rd week of February. The following was our itinerary—

<i>During the pre-winter months</i> (August to November)	<i>During the winter months</i> (December to February)
1 24-Parganas	17 Mymensingh
2 Darjeeling	18 Rangpur
3 Dinajpur	19 Jalpaiguri
4 Malda	20 Bogra
5 Murshidabad	21 Pabna
6 Nadia	Jessore
7 Burdwan	Nadia
8 Hooghly	Hooghly
9 Birbhum	22 Howrah
10 Bankura	Burdwan
11 Midnapore	Birbhum
12 Jessore	Murshidabad
13 Khulna	23 Faridpur
14 Barisal	24 Tippera
15 Chittagong	25 Noakhali
16 Dacca	Dacca

*Adults caught in traps*—We only used three “Tea-Box” traps at each place. They were usually set up in bath-rooms, out-houses, godowns, kitchen, etc., near the Dâk Bungalow in the town. They, however, usually attracted more culicines than anophelines at all the places, and sometimes, nothing but a large number of culicines. Of the “dangerous” species, we caught only *A. aconitus* and *A. culicifacies*.

*Some special points for notice*—(1) We collected and registered about 15,000 larvæ, small and large, in the 6 months’ survey, but only about 9,000 came up for examination, i.e., about 40 per cent were lost. This was probably due to the smaller falling prey to the larger, when they were unavoidably kept

# A NOTE ON THE DEGENERATION OF VACCINE LYMPH ON PASSAGE THROUGH THE SAME VACCINIFER \*

BY

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[Received for publication, April 29, 1927 ]

THE observations recorded in the following note are of some interest to those who prepare vaccine lymph in this country

Nejland's method of using alternate vaccinifers, the rabbit, cow and buffalo, for the production and preservation of a potent lymph was introduced into the King Institute, Madras in 1921, and met with unqualified success. The difficulty encountered in obtaining buffaloes, however, made a slight modification in the original process necessary, and the cow calf had to be used for the mass production of the lymph instead of the buffalo as originally used in Java.

One of the essentials of the process lies in the strict alternation of the animals, the same vaccinifer never being used more than once in each cycle. It was noticed in practice, however, that if subpassages were carried out continuously on calves, after having passed through the rabbit and buffalo, that the weight of pulp produced per calf in the 2nd and subsequent removes, was frequently greater than that obtained from the animals sown direct from the buffalo. It thus appeared as if the virus required some acclimatization to the cow calf before exhibiting its optimum growth.

An increase of yield is of economic importance if it can be shown that the potency of the lymph produced in this way does not suffer. An investigation was, therefore, undertaken to determine this point.

After the usual passage through the rabbit and buffalo, a number of different seeds were passed through six series of calves in succession. The removes were labelled from 1 to 6. The average amount of pulp produced, the vesiculation rate on calves and the vaccination rates on children were recorded and arranged according to the number of the passage. The results are set forth in detail in the Table.

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\* This work was carried out at the King Institute, Madras, during the year 1923

*A christophersi* typically lived in sluggish or fast-flowing streams with grassy edges, not higher up into the hills than about 1,000 feet, and it was never found in puddles. It was possible that this species had a more powerful flight than *A rossi*, *A metaboles* (*maculatus*) also bred in streams and in the swamps beside the streams such as rice-fields and small pools, this species also was not found in foul puddles, it bred at some distance from human habitations. *A fuliginosus* was found breeding in the same sort of places as *A nigerrimus* (and in the hills *A lundesayi* bred in the same position as *A metaboles*).

Stephens and Christophers (1902a) In a paper on classification of Indian Anophelines, these authors specified the typical breeding places of the above-mentioned species, their classification holding also for those others which have been subsequently found to be prevalent in Bengal, as follows — 'Open-water breeders,' i.e., those found in water with much aquatic vegetation, ponds, lakes, banks of rivers and swamps (*A barbirostris* and *A sinensis* (*nigerrimus*)) the habits of both these species were very similar and they were very common in Bengal. 'Stream-breeders,' found in swiftly running ditches and nullahs (*A culicifacies*, *A listoni*, *A christophersi*) (*A culicifacies* was found in irrigation ditches in Lahore and in the running water of nullahs near Nagpur. It was the common stream mosquito in those places and the adults were found plentifully in the rayats' houses). *A christophersi* was the common species in the Duars. *Pool-breeders*, (a) those selecting clean pools with green algæ, especially small pools left in river-beds (*A jamesi*, *A maculatus*, *A theobaldi*, *A lundesayi*), *A theobaldi*, the adults were stated to enter houses but were not common. The larvæ frequented especially sluggish streams with much growth of alga. *A jamesi* larvæ were found in only one situation, a chain of small muddy puddles with water-trickling through them. The adults were caught in adjoining habitations. *Pool-breeders*, (b) those selecting muddy-pools (*A rossi*, *A metaboles*\*) *A metaboles* was a rare mosquito in Bengal. Both it and *A rossi* larvæ were also found in pots and tins of water. Of the above species, *A nigerrimus* and *A barbirostris* but rarely entered houses and *A lundesayi* had never been found to do so †.

*fuliginosus* was found breeding in a weedy lake and the adults were in thousands in adjoining huts.

‡ Christophers and Bentley (1909), apropos of malaria in the Duars, presumed that a condition which they called residual anophelism, was to be found there, a condition which implied the maintenance of adult anophelines in some numbers long after their breeding places dried up. More specially they drew attention to the main anopheline breeding places of the tract, noting the great prevalence of *listoni*, *maculatus*, and *rossi*, and the presence of a few others *culicifacies* being rare.

\* *stephensi* ?

† One of us (C S) has several times lately caught it in houses at about 3,500 feet, and James and Liston also record it as caught in houses in the Himalayas.

‡ The Drainage Committee report (1907) has been unobtainable, so cannot be reviewed.

The lymph\* from 608 calves was used in the experiments. Following the usual custom each cup of lymph was the product of at least 5 calves so that a lymph of average potency was always assured. The details of the cups made from each seed were added together and the results pooled for each subpassage separately.

There is a definite increase in yield in the 2nd, 4th and 6th removes over the average (23.3 grms) and the 1st remove (23.1 grms). The vesiculation tests, as a rule, produced continuous lines and, where discrete vesicles were obtained, the rates were above those necessary to produce a satisfactory result. On children the case success rate was cent per cent until the 4th subpassage, after which it became irregular.

So far, then, there was no evidence of degeneration in the earlier removes at any rate.

The insertion success rate, however, showed a gradually increasing failure rate from the 1st to the 6th remove (0.41 per cent to 7.4 per cent) showing clearly that there was a definite and progressive, though slight, loss of potency with each subpassage.

It is evident, therefore, that if a lymph of the highest potency is desired Nejland's original process of strict alternation of vaccinifers must be adhered to, even although this procedure may mean a slight loss in the amount of lymph obtained per calf.

It is interesting to note also that these experiments show that weight of pulp by itself is not always a reliable guide to the potency of a lymph but that it should always be controlled by other tests of which the insertion success rate on children is the most important.

I must acknowledge my indebtedness to Khan Sahib Mohamed Omar Sahib, the senior vaccine assistant of the King Institute, Gundy, for undertaking the work of extracting the results from the Institute records and arranging them in the necessary form.

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\* For this test the lymph is diluted 1—500. A rate of 1 vesicle per linear inch sown will, as a rule, give a success rate of 90 per cent a child.

#### REFERENCE

CUNNINGHAM and CRICKSHANK      *Ind Jour Med Res*, Vol X, No 4, April, 1923



Strickland (1925) replying to his general thesis put in a plea that the problem should be studied entirely in terms of the mosquito

Stewart, Sui, and Iyengar (1925) also in connection with the Meenglas experiment studied the prevalence and breeding places of the anophelines in that part of the Duars

Covell (1927) summarised these and other records in his monograph on the geographical distribution of all the Indian species

Strickland, Chowdhury and others in a report on malaria in the Tea Gardens of the Duars (as yet unpublished) studied the same subject in great detail for the whole of that region, and recorded for the first time *maculipalpis* and *kochi*

It may also be mentioned here, that it is understood that the Public Health Department of the Province has in the past collected a large amount of information on this subject which has not yet been published

With regard to the anopheline work previously done in Bengal, one may justly conclude that considering the great importance of malaria in the Province, and the great attention it has received from epidemiologists, it is remarkable how little has been done, since Stephens' and Christophers' time, excluding of course the Duars. Perhaps the reason for this has been the tendency to follow Christophers' and Bentley's doctrines (1909) enunciated in their report on malaria in the Duars, which put a premium on the idea that it would be easier to deal with the human factor rather than the anopheline, in any campaign to deal with the trouble there. And this idea has been strongly reinforced by Fry (1912) and by Bentley (1925). Indeed, Fry said 'all species of anophelines found in Bengal, breed in similar situations and have similar habits' 'From the practical point of view it does not matter whether the *myzomyias* or *fuliginosus* are the carriers as they all breed in the same sort of places' 'All species known in Bengal breed alike in the innumerable bhils and water-holes' Bentley on the other hand advocated extensive irrigation schemes to neutralise the loss of the annual inundations in the malarious districts,

#### THE MOSQUITOES OF BENGAL FOUND IN OUR SURVEY

The species recorded by previous observers have been indicated shortly above, and those found by ourselves are for all practical purposes the same as will be seen in the table below

It may be mentioned here, however, that in addition to the species enumerated, we have lately obtained a specimen of *A. theobaldi* which bred out from a larva along with *A. rossii* and *Culex fatigans*. It was caught at Entally in Calcutta in a clean-water drain with grassy edges. We have also had *stephensi* from the hills at 3,500 feet

The species *A. jamesi* mentioned below in our tables is being described in a publication by us as a new species *A. pseudojamesi* and in view of the difficulty experienced by epidemiologists in the past in allocating the malaria of the Province to any specific cause, this find may prove of great importance

We have also taken *A. ludlowi*, which has been but rarely captured previously in Bengal, and *A. gigas* (var. *sumlensis*) which has never been, as yet taken in the area, the latter specimens sent to us by Dr Newman of Saidpur

# AN ANOPHELINE SURVEY OF THE BENGAL DISTRICTS.

BY

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AND

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[Received for publication, May 6, 1927]

WE have to thank Lieut-Col Fry, C I E , I M S , Professor of Hygiene at this Institute, and Lieut-Col Stewart, I M S , Offg Director of Public Health, Bengal, for assistance in the light of their experience of the Province, in formulating an enquiry, the result of which is here reported upon, for studying the anophelines of Bengal, and we also wish to thank the Secretary and Members, the Scientific Advisory Board and the Indian Research Fund Association for placing a grant at our disposal to carry out the work

## MATERIAL AND METHODS

The following notes have been compiled by the field staff —

' The Bengal Districts Malaria Survey, August 1926 to March 1927

*Staff for the field work —*

- 1 Chief Assistant-in-charge
- 2 Field-collector
- 3 Laboratory-assistant
- 4 Mosquito-catcher, (assistant to field-collector)
- 5 Peons

*Period of work* — 19th August 1926 to 19th February 1927 (= 6 months)  
The staff took holidays during the Pujahs and Xmas There was a poor collection during the first fortnight, the training period

- (2) The enormous increase in the Duars of the *rossi-vagus* group
- (3) The relatively enormous numbers in the Province of *A. sinensis*, *A. barbirostris* and *A. fuliginosus*
- (4) The increase in the Province of *A. aconitidis* to about the same rate as *A. funestus* decreases. If one lumps these species together the net result is about the same in the Duars as for the rest of the Province. We did not examine to any extent the Duars 'jampoos' (or rice-field irrigating-channels) during the rains, where it is possible that *A. aconitidis* might have been found in numbers corresponding to those in the Province.
- (5) The approximately equal rate of *A. culicifacies* in the Duars and the Province
- (6) The almost complete lack of *A. stephensi* although it is a common Calcutta mosquito (but wells were not examined except in Krishnagar)
- (7) The considerable numbers of *A. pseudojamesi* in the Province

#### THE RELATIVE LOCAL PREVALENCE OF THE DIFFERENT SPECIES IN BENGAL

The figures which we obtained in our Survey are submitted in the following tables (1) the total numbers of larvæ (Tables I to III), (2) the per cent of each species as larvæ to the total larval catch (Table IV), (3) the total numbers of adults (Table V), but the last mentioned records are unfortunately too slight to be of much value.

We wish to draw attention to the Table IV species percentages for the three great administrative divisions, North West and East Bengal. Before doing so, however, we bear in mind that the North in physical characteristics is coloured by those of the Terai and the Duars, which implies that denudation of the land surface is the predominant factor in the local geography, whereas in Lower (West and East) Bengal deposition of the delta overshadows denudation of the land-surface. As far as that part of the North called the Duars and the rest of the Province was concerned, the resultant effect on anophelism was the subject of Stephens' and Christophers' (1902) important paper already referred to and of the analysis hereabove made by ourselves.

If now we combine the data for East and West Bengal given in the tables and compare them with those for North Bengal we get the following result —

Bengal	<i>sinensis</i> .	<i>barbirostris</i>	<i>fuliginosus</i>	<i>vagus</i> or <i>rossi</i>	<i>aconitidis</i>	<i>funestus</i>	<i>maculatus</i>	<i>culicifacies</i> .	<i>Focke</i>	<i>Indlowi</i>	<i>tesselatus</i>	<i>jamesi</i>	<i>pallidus</i>	<i>pseudojamesi</i>	<i>maculipalpis</i>	<i>stephensi</i>
North	40.95	9.7	28.6	19.5	3	1	2	2	0.5			3		0.5		
South (East-West)	40	9	25	15.4	4.7	2		3.3	*	1	*	3	2	1.5	*	*

\* Means the percentage was a very small one

together to make the number of the breeding-bottles within the limit of management on our frequent journeys

(2) Some of the towns of Bengal (viz, Krishnagore, Dinajpur and Jessore), were said to be more malarious than the country-around, due perhaps to the fact of *A stephensi* breeding more readily in towns than in the country. We caught *A stephensi* at Krishnagore town'

#### THE OBJECT OF THE SURVEY

The scheme of work reported on here was designed to obtain an indication of a differential local prevalence of the anophelines in Bengal which might account for the well-known local variations in malarial incidence in the Province, and ran on parallel lines to Stephens' and Christophers' work reported to the Royal Society (1902) on 'Malaria Endemicity in its relation to species of Anophelines'. At the same time, no attempt will now be made to correlate our findings with any malaria records, partly because since Stephens' and Christophers' time it has transpired that these are by no means so simple as their work led them to suppose

As justification for this investigation the following commentary from the *Lancet* may be cited 'Extraordinary effects have followed intensive study of the conditions which favour the breeding of the particular anopheline species locally implicated. It is becoming widely held elsewhere that identification of implicated species promises well for future preventive work. No later work on these lines than Fry's is traceable in Bengal and it is difficult to believe that species with identical habits are implicated in the different conditions of West and East Bengal'

#### PREVIOUS MOSQUITO RECORDS IN BENGAL

In the course of the papers of the authors referred to beneath there have been notices of anophelines in Bengal from one point of view or other. These records will here be shortly recorded

Stephens and Christophers (1902) noted the large numbers of anophelines in Lower Bengal and their comparative scarcity in the Duars. In Calcutta, *A rossi* was present in enormous numbers, in the plains generally *A rossi*, *A fuliginosus*, and *A sinensis* sub sp *nigerrimus*, while in the Duars, *A metaboles* (= *maculatus*) and *A christophersi* were the predominant species. In the Bengal hills (with which we have not here concerned ourselves), there were *A rossi*, *A lundsayi*, and *A metaboles*

They noted that *A nigerrimus* bred extensively over large areas of marsh land, but was rarely caught in the adult state in houses. *A fuliginosus* was more abundant in houses

In another paper giving the distribution in more detail, the habits and breeding places were noticed. *A rossi* was essentially a domestic and foveal species, *A nigerrimus* on the other hand was found in sluggish streams, river banks, marshes, and swampy pools, a deep clean stagnant ditch bred *A nigerrimus*, while shallow foul puddles alongside bred *A rossi*.

TABLE I—(contd.)  
Numbers of Larvæ caught

Divisions	<i>sinensis</i>	<i>barbrosistris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossi-vagus</i>	<i>acanthus</i>	<i>funestus</i>	<i>maculatus</i>	<i>culicifacies</i>	<i>koehn</i>	<i>indlowi</i>	<i>tesselatus</i>	<i>janessii</i>	<i>paludus</i>	<i>janessii</i>	<i>maculipal-</i>	<i>stephensi</i>	Total
Rajshahi	380	209	615	323	86	12	6	2	4	4	1			6			1		2,149
Presidency	532	170	415	245	103	12	7	8		8				3	10			1	1,515
Burdwan	628	133	565	194	132	18	58	2		208				1	1		3	1	1,997
Dacca	998	246	414	109	6	8	68				1	5		5					1,871
Chittagong	469	63	253	170	3	15	177	2				1	3	12	14				1,207
	3,507	821	2,264	1,041	330	64	316	14	4	220	2	6	3	27		101	3	2	8,739
North Bengal	880	209	615	323	86	12	6	2	4	4	1			6	13				2,149
West Bengal	1,160	303	980	439	235	29	65	10		216				4	1		3	2	3,512
East Bengal	1,467	309	669	279	9	23	245	2			1	6	3	17	14				3,078
	3,507	821	2,264	1,041	330	64	316	14	4	220	2	6	3	27	14		3	2	8,739
Bengal Presidency	3,507	821	2,264	1,041	330	64	316	14	4	220	2	6	3	27		101	3	2	8,739

James and Liston (1911) in their Monograph noted as Bengal species —	
<i>atkeni</i> (Duars)	<i>rossi</i>
<i>indesayi</i> var <i>maculata</i> [Kurseong]	<i>ludlowi</i> (Fraserganj and Sunderbans)
<i>culcifacies</i>	<i>willmori</i> var <i>maculosa</i> [Kurseong]
<i>listoni</i>	<i>stephensi</i> (Calcutta)
<i>maculatus</i> [Kurseong] (Duars)	<i>barbirostris</i>
<i>fuliginosus</i> (plains) [Kurseong]	<i>sinensis</i>
<i>jamesi</i>	<i>elegans</i> (Duars)

Of the habits of the adults and larvæ of these species, they noted the following —

'*culcifacies* is a markedly domestic species and apparently feeds almost entirely on human beings. They are rather difficult to catch in houses, as they hide very successfully in the darker corners, their attitude when resting, being culex-like'

'*listoni*, usually breeds in running streams and the adults are strong fliers'

'*fuliginosus* is also a strong flier, the larvæ being usually found in shady natural ponds or tanks, with grass and weed at the edges'

'*rossi* breeds in rain-formed pools, and rice-fields usually not far from houses. The larvæ have been found in water heavily contaminated with sewage and the adults are essentially "domestic"'

'*stephensi* a "domestic" species, breeding not far from habitations, its larvæ being very catholic in their tastes, breeding in almost every sort of collection of water'

'*barbirostris*, the larvæ are usually found in shady pools with much vegetation or in tanks, overgrown with weeds. It is not a "domestic" species'

'*sinensis*, the larvæ are usually found in deep shady pools at some distance from houses'

Fry (1912), (1914) drew attention to the difficulty of finding larvæ or adults in the Terai. In his reports there were many references to anophelines in their relation to the epidemiology of malaria in Bengal, [but here we are not touching this side of the subject]. Fry found mosquitoes scarce in September and plentiful in December. He also drew attention to the universal distribution of *A. fuliginosus*, but all species 'breed in the same sort of places' and again 'all species in Bengal breed alike in the innumerable bheels and water-holes'. Drs. Sen and Mitra's report on some mosquito-surveys was included in Fry's reports, also one of Dum Dum by Dr. M. C. Ghosh.

Bentley and other officers of the Public Health Department (1918 *et seq.*) with regard to the antimalarial scheme of operations at Meenglas in the Duars, made many references to the prevalence of the species and their breeding places.

Bentley's (1925) report 'Malaria and Irrigation in Bengal' was an attempt rather to prove that malaria in Bengal was due to the decaying state of the agricultural industry, but in general he thought that this was associated with greater facilities for anopheline breeding because of the loss of the perennial inundations which are inhibitory to anophelism.

TABLE II—(contd.)  
Presidency Division

District and Centre	sinensis	barbrosistris.	fuliginosus	vagus	rossi	rossi-vagus	acutus	funestus	maculatus	chilifacies	janessii	pallidus	janessii x	stephensi	TOTAL
24-PARGANAS—															
Calcutta	32	10	2	3	14	1	2	8							72
MURSHIDABAD—															
Berhampur	68	60	31	52	47					1					259
Bazar Sohu			51	1						7					59
NADIA—															
1st visit 19 to 24-9-26															
Krishnagar	54		17	115	35	11	.				..			1	233
2nd visit 8 to 11-1-27	87	31	116	23		.	2				3		3		266
JESSORE—															
Jessore	119	36	40	24			2						1		220
Bongaon	83	1	129	1								2			218
KHULNA—															
Khulna	89	32	29	26	7		1						4		188
	532	170	415	245	103	12	7	8		8	3	3	8	1	1,515

Summary for Division—1,515

In our table below, we have analysed the differential anopheline fauna (larvæ) *vis-à-vis* the Duars which has been the scene of so much work \*

*Anopheline Larvæ*

	THE DUARS TEA GARDEN SURVEY April 15—July 15, 1926		THE BENGAL MOSQUITO SURVEY Aug 16—Feb 16, 1926	
	Actual	Per cent	Actual	Per cent
<i>rossi</i> and <i>vagus</i>	1,618	72.40	1,435	16.4
<i>maculatus</i>	259 †	11.59	4	0.5
' <i>minimus</i> ' and ' <i>listoni</i> '	115	5.15	14	0.2
<i>acomtus</i>	0		316	3.6
<i>culticifacies</i>	63	2.82	220	2.5
<i>tessellatus</i>			3	0.03
<i>sinensis</i>	60	2.68	3,507	40.1
<i>fuliginosus</i>	60	2.68	2,264	25.9
<i>pallidus</i>			14	0.17
<i>jamesi</i>	48 ‡	2.15	27 ‡	0.34
<i>pseudojamesi</i>			101	0.17
<i>kochi</i>	5	2.2	2	0.02
<i>barbirostris</i>	4	1.8	821	9.40
<i>karwari</i>	2	0.9		
<i>leucosphyrus</i>	1	0.4		
<i>maculipalpis</i>	0		3	0.03
<i>stephensi</i>	0		2	0.82

This comparison between the Duars and the whole of the Presidency brings to light —

(1) The great numbers in the Duars of *A. maculatus*, † *A. funestus*, ‡ (*listoni* and *minimus*) and *A. maculipalpis*

\* The different seasons, however, of these two surveys should be noted

† Must have included some *maculipalpis*, the adults of which were quite common

‡ *pseudojamesi* not included



TABLE II—(contd.)

## Dacca Division

District and Centre	sinensis	barbivittatus	fuliginosus	vacuus	rossi	rossi-vaguus	acutus	bachii	janseui	indlorum	paludus	janseui	Total
BAKHARGANJ— Bansal	118	171	52	13			11			5			370
DACCA— 1st visit 20th to 24-11-26	170	16	89	18		1	14	1				1	210
2nd visit 13th to 15-2-27	275		88	17			30						410
Manikganj	35	5	37	24	1	5	1						108
MYMENSINGH— Mymensingh	250	36	41	22	1				7			2	359
FARIDPUR— Faridpur	150	18	107	15	4	1	12		4		1	2	314
	998	246	414	109	6	8	68	1	11	5	1	5	1,871

Summary for Division—1,871

TABLE I  
Numbers of Larvæ caught

Districts	sinensis	barbistris	fuliginosus	vagus	rossi	rossi-vagus	acanthus	funestus	maculatus	culicifacies	koehli	pulcherrimus	indorum	tessellatus	janseus	palhdus	janseus	maculipalpis	stephensis	Total
Darjeeling	42	15	11	5	10	11		2	1	3	1				3					85
Jaipalguri	138	4	180	137	46				2											526
Dinapur	113	5	20	13	5	1			1											156
Rangpur	174	48	93	3	18					1							1			320
Malda	88	5	20	18	1		2													153
Bogra	295	58	170	52	1		4								1					581
Pabna	30	74	121	95	6										2					328
Murshdabad	68	60	82	53	47															318
Nadia	141	31	133	138	35	11	2			8					2	1			1	499
Jessore	202	37	169	25			2								3	2				438
Khulna	89	32	29	26	7		1								1					188
24-Parganas	32	10	2	3	14	1	2	8												72
Birbhum	42		97	2	17	3		1		48					1	6		1		217
Burdwan	336	60	370	152	100	15	18			160					1	3		2		1,262
Hooghly	171	54	72	12			40	1												351
Howrah	34	1	3																	38
Midnapur	35	16	23	23	15															112
Bankura	10	2		5																17
Barisal	118	171	52	13			11						5							370
Faridpur	150	18	107	15	4	1	12								2	1				314
Dacca	480	21	214	59	1	6	45				1				1		4			828
Mymensingh	250	36	41	22	1										2		7			359
Chittagong	197	59	141	48		2	13	2					1	3						473
Noakhali	63	2	18	1			50													176
Tipperra	209	2	96	121	3	13	114								5		37			558
	3,507	821	2,264	1,041	330	64	316	14	4	220	2		6	3	27	14	101	3	2	8,739

TABLE III  
Numbers of Larvæ caught

Centres	sinensis	barbivittatus	fulginosus	vagus	rossi	rossi-vagus	acanthus	funestus	maculatus	culicifacies	koehli	pulcherrimus	indlowi	tessellatus	jamesi	paludosus	jamesi	maculipalpis	stephensi	Total
Calcutta	32	10	2	3	14	1	2	8	1		1									72
Siliguri	42	15	11	5	10															85
Dinajpur	113	5	20	13	5		2			1										156
Malda	88	5	20	18	18					1										153
Berhampur	68	60	31	52	47					1										259
Krishnagar—																				
1st visit	54		17	115	35	11	2													233
2nd "	87	31	116	23	34															266
Nabadwip	82	27	83	53																280
Hooghly—																				
1st visit	26	54	23	12		8	40	1												115
2nd "	145	28	49	65			2													236
Burdwan	79		36	2																117
Bolpur	1		3	5																17
Bankura	10	2																		112
Midnapur	35	16	23	23	15															220
Jessore	119	36	40	24	7		1													188
Khulna	89	32	29	26																370
Barisal	118	171	52	13			11													473
Chittagong	197	59	141	48			13													310
Dacca—																				
1st visit	170	16	89	18		1	14				1									410
2nd "	275		88	17			30													359
Mymensingh	250	36	41	22	1															320
Rangpur	174	48	93	3		1			1											526
Jalpaiguri	138	4	180	137	46	11	4	2	2	3										581
Bogra	295	58	170	52	1															328
Pabna "	30	74	121	95	6															218
Bongaon	83	1	129	1			2													38
Belur	34	1	3																	96
Memari	14		38		2		2													189
Kalna	105	4	54	8	1		14													110
Katwa	46	1	60																	59
Bazar Sohul			51	1																369
Asansol	10		99	26	63	7														211
Suri	41		94	17	17	3														314
Faridpur	150	18	107	15	4	1	12	1												558
Comilla	209	2	96	121	3	13	114													176
Noakhali	63	2	37	1			50													108
Manikganj	35	5		24	1	5	1													
	3 507	821	2 264	1 041	330	64	316	14	4	220	2		6	3	27	14	101	3	2	8 739

TABLE II  
Rajshahi Division

District and Centre	<i>sinensis</i>	<i>barbistris</i>	<i>fulginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossi-vagus</i>	<i>acomis</i>	<i>funestus</i>	<i>maculatus</i>	<i>cultifacies</i>	<i>koehn</i>	<i>pulcherrimus</i>	<i>jamesoni</i>	Total
DARJEELING—														
Siliguri	42	15	11	5	10				1		1			85
DINAJPUR—														
Dinajpur	113	5	20	13	5	.								156
MALDA—														
Malda	88	5	20	18	18	1.	2			1			1	153
RANGPUR—														
Rangpur	174	48	93	3		1			1					320
JALPAIGURI—														
Jalpaiguri	138	4	180	137	46	11		2	2	3			3	526
Bogra—														
Bogra	295	58	170	52	1	..	4		.				1	581
PAHNA—														
Pabna	30	74	121	95	6	..							2	328
	880	209	615	323	86	12	6	2	4	4	1		6	2,149

Summary for Division—2,149

TABLE IV—(contd.)  
Percentage of Larvæ caught.

Divisions	<i>sinensis</i>	<i>barbrosistris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rosini</i>	<i>acutus</i>	<i>funestus</i>	<i>maculatus</i>	<i>culicifacies</i>	<i>koehli</i>	<i>indlowi</i>	<i>tessellatus</i>	<i>jamesi</i>	<i>paludus</i>	<i>jamesi</i>	<i>maculipalpis</i>	<i>stephensi</i>	<i>rossi-vagrus</i>
Rajshahi	40.9	9.8	28.6	15.0	4.0	3	1	2	2	0.5			3			0.5		12
Presidency	35.0	11.3	27.4	16.3	6.8	4.5	5		5				2	2		5	0.5	8
Burdwan	31.4	6.7	28.3	9.7	6.6	2.9	1		10.4	0.6			0.5	5	2.20	2	0.5	9
Dacca	53.3	13.2	22.2	5.8	3	3.6					28		28	0.6	5.2			4
Chittagong	38.9	5.2	21.1	14.1	2	14.7	2			0.5	1	2	10		3.1			12
North Bengal	40.95	9.7	28.6	15.0	4.0	.3	1	2	2				3		0.5			55
West Bengal	33.05	8.65	27.9	12.5	6.7	1.8	3		6.14	0.3			1	4	1.5	0.9	0.7	8
East Bengal	47.6	10.05	21.75	9.15	3	7.85	0.7			0.2	20	1	5.5	0.3	1.57			75
Bengal Presidency	40.1	9.4	25.9	11.9	3.8	3.6	2	0.5	2.5		0.7	0.3	3.4	1.7	1.7	0.3	0.2	7

TABLE II—(contd)  
Burdwan Division

District and Centre	<i>sinensis</i>	<i>barbrostus</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>rossi-vagus</i>	<i>acomis</i>	<i>funestus</i>	<i>culicifacies</i>	<i>maculipalpis</i>	<i>stephensi</i>	<i>jamesi</i>	<i>indlowi</i>	<i>pallidus</i>	<i>jamesi</i>	Total
BURDWAN— Nabadwip	82	27	83	53	34											279
Burdwan	79	28	36	65		8	2									218
Memari	165	5	152	8	3		16						2			395
Kalna	10		99	26	63	7			160	2	1	1		1		369
Asansol																
HOOGHLY— 1st visit																
1st to 6-10-26																
Hooghly	26	54	23	12												115
2nd visit	145		49				40	1						1		236
13, 14 & 16-1-27																
BIRBHUM— Bolpur	1		3	2		3										6
Suri	41		94		17			1	48	1				6		211
BANKURA— Bankura	10	2		5												17
MIDNAPUR— Midnapur	35	16	23	23	15											112
HOWRAH— Belur	34	1	3													38
	628	133	565	194	132	18	58	2	207	3	1	44	2	8	1	1,997

Summary for Division—1,997

TABLE V—(contd)

*Bengal malaria mosquito survey Schedule of adult mosquitoes caught in various places*

Divisions	<i>sinensis</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>rossi-vagus</i>	<i>culicifacies</i>	<i>maculatus</i>
Rajshahi	6	2	5	3	8		
Presidency	6	13	114	13	24		
Burdwan	3	8	34	7	3	3	1
Dacca							
Chittagong	2						
	11	23	153	23	35	3	1
=249							
North Bengal		2	5	3	8		
West "	9	21	148	20	27	3	1
East "	2						
Bengal Presidency	11	23	153	23	35	3	1
GRAND TOTAL					=249		

We remark about the table on page 384 in the first place, that if we use the prevalence of *A. maculatus* as an index of 'high-land' conditions and *A. sinensis* of the converse, we see that the records for North Bengal are *not so highly* coloured by the Duars characteristics as we supposed would be the case. That being so, we would not expect to find a great difference between Upper and Lower Bengal mosquitodora in general. Actually, there is a remarkable correspondence between the figures we have obtained for *A. sinensis*, *A. barbirostris*, *A. fuliginosus* and the *rossi-vagus* combination, all these species comprising about 94 per cent of the total catch. The same correspondence also exists in respect to the rest of the commonly accepted malaria-carriers in North Bengal as compared with Lower Bengal. This does not bear out the indication (although this was not too decided) given in the comparison between the Duars and the rest of the province, and may be due to the fact that those parts of North Bengal, other than the Duars, having an anopheline fauna very distinct from the Duars, give the nett result for the North\* as stated

\* There may be no significance in the slight rise in the *A. fuliginosus* and *vagus-rossi* rate in the North, but we will analyse this matter further below.

TABLE II—(concl'd)  
Chittagong Division

District and Centre	sinensis	barbrosistris	fuliginosus	vagus	rossii	rossi-vagus	acanthus	funestus	hidlowi	tesselatus	jamesi	jamesi x	Total
CHITTAGONG— Chittagong	197	59	141	48		2	13	2	1	3	7		473
TIPPERA— Comilla	209	2	96	121	3	13	114						558
NOAKHALI— Noakhali	63	2	18	1			50				5	37	176
	469	63	255	170	3	15	177	2	1	3	12	37	1,207

Summary for Division—1,207



stagnant with weeds, anophelines are usually found. Khals are also seen with bare edges and full of muddy water, and no larvæ are found in such. Some khals are not permanent, and dry up in the cold weather.

*Small streams*—Generally full of clear slow moving water. Larvæ are mostly to be found in bays in such streams or at any point where the water has been dammed. A few very small streams running fast and containing clear spring-water were examined and yielded anophelines.

*Drains, kutcha and pucca*—In pucca drains having a brick or concrete invert and containing running-water larvæ are not found. The inverts, however, tend in course of time to become covered with earth, and if neglected, weeds commence to grow in them. The drains then become favourable breeding places. Kutcha drains with slow-moving or stagnant water, and grassy or weedy edges nearly always contain larvæ.

*Irrigation canals*—Found generally by sides of paddy-fields, the water in these canals is usually stagnant and harbours anophelines.

*Dead or dying rivers*—These are to be found all over Bengal, and in most of them larvæ can be found. Some of the rivers are quite healthy in parts, but other portions of the same river are stagnant and dead. Rivers have been found to be quite covered with hyacinth at one point and quite free from any hyacinth at another point, a few miles distant. There are rivers with stagnant water and hyacinth and weeds at the edges whilst the water in the centre of the river is moving steadily. Such rivers generally contain anophelines. Many rivers have been found with a strong flow and with bare sandy edges. These rarely reveal larvæ.

*Swamps*—The depth of water varies, but anophelines can almost always be found in pools here and there. Conditions are favourable to the breeding of anophelines, these swamps being almost always full of clean water, grass and weeds.

*Bhils*—These vary considerably in size, also in appearance, many dry up rapidly, particularly if small in size, and most have some growth in the water whether at the sides only or over the entire surface. In some bhils, the growth is so dense and so high, that the water is not visible except at close quarters. Anophelines and culicines are almost always found in bhils.

*Ponds*—Temporary and permanent, the former drying up during the cold weather. Generally found near villages and used by the people in much the same way as tanks. The ponds usually have bare edges with clear or muddy water, and are not often covered with weeds. Larvæ are not usually found in them.

*Pools*—Mostly rain-water and temporary, found in almost any place where there is a depression in which water can collect. There pools are often full of grass, and contain anophelines.

*Pools, in river beds*—Found on *char* land and varying in shape and size. Water is clear, edges sandy, anophelines are often found in great numbers, although there may be no growth in the water and the edges are bare. On *char* land water which collects round the pillars of bridges almost always contains anophelines. Very small pools about a foot in diameter only, would be found to be full of larvæ whilst much larger pools situated close to these contained no larvæ.

TABLE IV  
*Percentage of Larvæ caught*

Districts	<i>sinensis</i>	<i>barbivirostris.</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi-vagus</i>	<i>aconitus</i>	<i>funestus</i>	<i>maculatus</i>	<i>cultivacies</i>	<i>koehi</i>	<i>pulcherrimus</i>	<i>indlowi</i>	<i>tesellatus</i>	<i>jamesi</i>	<i>palidus</i>	<i>jamesi v</i>	<i>maculipalpis</i>	<i>stephensi</i>
Darjeeling	494	176	129	59	118			12		12			.	6				
Jaipaguri	262	8	342	260	87	21	4	4	6									
Dinajpur	724	32	128	84	32													
Rangpur	544	150	290	10		3		3	6					6				
Malda	575	33	131	118	118													
Bogra	508	100	292	90	2	13								2				
Pabna	91	226	369	290	18	6								6				
Murshidabad	214	188	258	167	148				25									
Nadia	283	62	266	277	70									6	2			
Jessore	461	85	386	57		4								4	4			
Khulna	474	167	155	139	38	4												
24-Parganas	445	139	28	42	195	5	110											
					14	27												
Birbhum	193		447	9	78		5							1	28		5	1
Burdwan	266	47	293	120	79	14			221						2		2	
Hooghly	487	153	205	33	12	15	4		127						4			
Howrah	895	26	79			114												
Midnapur	313	143	205	205	134													
Bankura	588	118		294														
Barisal	319	462	141	35		30						13						
Faridpur	478	58	340	48	13	38								6	3	13		
Dacca	579	25	258	71	2	54				2				2				
Mymensingh	694	100	115	63	3									6		19		
Chittagong	417	125	298	101		28	4											
Noakhali	358	11	102	06	06	284							6	15		210		
Tippura	375	04	168	217	24	206								29				

TABLE VI  
*A sinensis*

Districts	Khals	Drains, kutchas.	Drains, pucca	Streams	Irrigation canals	Dead rivers	Bhils	Tanks no hyacinth	Tanks with hyacinth	Ponds	Swamps	Borrowpits small	Borrowpits, long	Ditches	Rice-fields	Jute-fields	Pools, temporary	Pools permanent	Drainage channels	Wells	Total
Darjeeling				5		21		20	32		2	13	32		13	4	10				42
Jalpaiguri				4		8		20			25	3									138
Dinajpur				14		15		19			12					6	32				113
Rangpur	43					23	10	6	8		42		4		17		44				174
Malda							30	35	15	2	11	3	49	6	5	1	11	21			88
Bogra				12						6	65	6	10	38			4				295
Pabna											12			4	5						30
Murshidabad				6				14		6	6										68
Nadia	13				2	4	29		6	2		20	30	24	12		35				141
Jessore	11					29	43		20		10		15	9	12		43	10			202
Khulna		14							10	6	4		4	9		5	66				89
24-Parganas				1					2				1								32
Birbhum							9	11	16				4				1				42
Burdwan		7				22	38	36	26	5	58	35	42		16		24	49			336
Hooghly					6			47	1	2	15		59		3		12		4		171
Howrah								34													34
Midnapur		10						4			10				6		11				35
Bankura																				4	10
Barisal		5																			118
Faridpur	6						22	13	7		23	25	51	29	23		16	6			150
Dacca	94					1		18			140	38	65	32	17		32				480
Mymensingh	6			6		1		25	36		26	29	86	36	25		12				250
Chittagong	4			2				11	68			29	11	25	24		34				197
Noakhali								10	45		89	8	8		11						63
Tippura	9			7		2		23	60												207
	186	36		57	8	126	181	346	365	29	550	232	471	234	189	16	387	86	4	4	3,507

TABLE V.

*Bengal malaria mosquito survey Schedule of adult mosquitoes caught in various places*

1926-27 Month	Districts	<i>sinensis</i>	<i>fulig- inosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossi- vagus</i>	<i>culici- facies</i>	<i>acomi- tus</i>
September	Dinajpur					6		
December	Jalpaiguri							
"	Rangpur		2	3				
"	Bogra					2		
"	Pabna			2	3			
September	Murshidabad		1	1	1	17		
"	Nadia		7	77	12	7		
January	"		3	25				
October	Jessore		2	4				
November	Khulna	6		7				
January	Birbhum	2		2				
October	Burdwan		5	8	1	1		
January	"	1	3	4	1		3	
October	Hooghly			19	5			1
January	"			1		2		
October	Midnapur							
November	Barisal							
February	Faridpur							
November	Dacca							
February	"							
November	Mymensingh							
"	Chittagong	F 1 M 1						
February	Tippera							
	TOTAL	11	23	153	23	35	3	1-

TABLE VIII  
*A. fuliginosus*

Districts	Nullas	Khals	Drams, kutcha	Drams, pucca	Streams	Irrigation canals	Dead rivers	Bhils	Tanks no hyacinth	Tanks with hyacinth	Ponds	Swamps	Borowpits, small	Borowpits, long	Ditches	Rice-fields	Jute-fields	Pools, temporary	Pools, permanent	Drainage channels	Wells	Total
Darjeeling					10		24		61		41	3	4	17				4				11
Jalpaiguri									2			2	25	17				2				180
Dinajpur					11		8			1			13	54				16				20
Rangpur									29	8		2	47	42					6			93
Malda					30		6					12	12	10								20
Bogra							73					12			10			16				170
Pabna																						121
Murshidabad				5			1		46		6	8	3	4	5							
Nadia		30					25	40														82
Jessore		13					72	47	12	10		5				3		12		15		133
Khulna																		8		9		169
24-Parganas											2			2				15				29
Birbhum	1																					2
Burdwan		11			6			14	29	11	4	83	3	33				3				97
Hooghly								11	29	42			23	80	10	4		31	62			370
Howrah								4	2	6				19				4				72
Midnapur			4						3													3
Bankura									10									9				23
Barisal																						
Faridpur		2					38	8	17			5	15		10	4		6				52
Dacca		52					1		54			59	6	23	19			19	8			107
Mymensingh		4			5				24	7		2		17	3	2		1				214
Chittagong																						41
Noakhali		7				1			10	88			25	7		8		12				141
Tippura									33	2		22	8	8		24						18
																						96
	1	119	4	5	62	3	249	132	361	178	53	262	125	333	60	47		153	100	12		2,264

On the other hand, the reason may be simply due to the collected data being insufficient. The matter needs further enquiry.

If now we compare the East and West Bengal record in the tables we see an increase more or less marked of *A sinensis* and *A barbirostris* in the East, while *A fuliginosus* and *rossi-vagus* are more markedly increased in the West. Of the two latter species, *A rossi* was distinctly rare in East Bengal.

Coming now to the rarer species, we obtained a very much larger number of *A aconitus* in East Bengal than in West, a fact quite in keeping with the 'moribund' state of the waterways in the West as compared to the East, but to counter-balance this *A culicifacies* was found in very much larger numbers on the West than on the East\*. Our new species *A pseudojamesi* was apparently equally prevalent in the two regions.

### THE ANOPHELINE BREEDING PLACES IN BENGAL

In order to do full justice to the observations made in our survey, we would have to analyse the physical conditions of each region, and we wished to do this in terms of the development of the delta and all that it implies and not in those of loose and popular phraseology. The former procedure would, however, have involved a much more prolonged study than we have had time to give to it, it being an extraordinarily complicated matter, of which only the fringe is touched by Medlicott in the paper of which an extract is appended (page 422). That being so, we are driven to submit our detailed findings in those very terms which really mean next to nothing, but as far as possible, we define them as follows —

#### SOME DEFINITIONS †

*Tanks* — From enquiries made, the average age of tanks is between 15 and 20 years, but this cannot be taken as being altogether reliable. They may be roughly divided into two classes, those containing hyacinth and water-weeds, and those which contain no growth of any kind. Experience has shown that tanks quite choked with hyacinth do not readily yield larvæ. Also larvæ are not usually found in tanks which have bare edges, and no growth in the water. In some places tanks were found to be full of foul water, giving off an offensive smell, and full of weeds and hyacinth, culicines were found in such tanks but no anopheles. Tanks containing clear or slightly muddy water, grassy or weedy edges, and with weeds, slime and some hyacinth at the sides, will almost invariably contain anophelines. Some tanks by the side of rivers are flooded out when the river rises, whilst the water in other tanks, rises only during the rains, and the tanks are never cleared out.

*Khals* — Artificial or otherwise, found in most cases to be full of clear stagnant water. The artificial khals are made for various purposes such as draining-water from bhils or are natural channels connecting two rivers. Where the water is

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\* If these two species are the main ones concerned in Lower Bengal malaria, then it is interesting to remember the comparative malarial incidence in the two parts.

† These notes are compiled by the field staff.

TABLE X

A rossu

Districts	Nullahs	Khals	Drains, kutcha	Drains, pucca	Streams	Irrigation canals	Dead rivers	Bhils	Tanks no hyacinth	Tanks with hyacinth	Ponds	Swamps	Borrowpits, small	Borrowpits, long	Ditches	Rice-fields	Jute-fields	Pools, temporary	Pools, permanent	Drainage channels	Wells	Total
Darjeeling									3		38		5				1	9				10
Jalpaiguri																						46
Dinajpur																						5
Rangpur																						
Malda											4											18
Bogra											1											1
Pabna							5												3			6
Murshidabad																						
Nadia		2	25	6	4				2		6	8	9					2				47
Jessore			4						4									6				35
Khulna			1																			7
24-Parganas											3			7	9		1					14
Birbhum																						
Burdwan							4															
Hooghly		2	4		1					4	3	18	7	6	8			38	8	14		17
Howrah																						100
Midnapur																						
Bankura			7													8						15
Barisal																						
Faridpur																						
Dacca					1				1										4			4
Mymensingh																						
Chittagong																						
Noakhali																						
Tippura					3																	3
		4	41	6	9		9		10	4	55	26	21	22	17	8	4	61	29		4	330

*Ditches*—With clear or muddy-water, running or stagnant, with grassy or bare edges. Generally by the side of roads. Those containing clean stagnant or slow moving water with grassy edges, generally yielded anophelines.

*Small borrowpits*—These are to be found everywhere by the side of railway-lines, roads, and inside villages. In each case, earth is taken out to build up the embankment or road, or to build houses, the pits made later filling up with rain-water. These borrowpits vary in size some being anything up to about ten feet square, and others no more than a foot square. In some districts, the water in these pits was quite muddy, and none produced larvæ when examined. Water in these borrowpits is generally clean, and larvæ can almost always be found in them if the edges are grassy or weedy. In many areas the borrowpits had bare edges and no weeds in the water. Larvæ were not generally found in such pits.

*Long cuttings*—Almost always found by the side of railway lines, and sometimes anything up to fifty or more feet in length. These cuttings are nearly always quite full of weeds, and water hyacinth, and usually contain anophelines. Cuttings full of muddy water and bare edges, rarely contain larvæ.

*Paddy-fields*—Generally contain water, though not to any depth. Larvæ are usually found, but there does not appear to be any special condition required, provided the water is clean. The larvæ are usually to be found round about the rice plants and not always at the edges of the field. Slime is sometimes found in the water and larvæ are more readily obtained if such is the case.

*Jute fields*—The same remarks apply here more or less. Later in the season jute is put into pools of water to steep. Only culicine larvæ were found in such pools.

*Tree holes*—Generally bamboo—these fill up with rain-water and yielded only culicines.

The different breeding places of each species according to the above descriptions are shown in the Tables VI to XVI and alternately, the various species found in each important type of breeding place is given in Tables XVII to XXIX.

The relatively large numbers of *A. acontus* found in dead or dying rivers, whilst in bñils, their physiographical end-product, there were relatively few, is interesting. In tanks free of water-hyacinth, there were about 0.3 per cent and in tanks with water-hyacinth about 10 per cent of this species.



TABLE XII  
*A. aconitum*

[illegible]

TABLE VII

*A. barburossus*

Districts	Khals	Drams, kutcha	Drams, pucca	Streams	Irrigation canals	Dead rivers	Bhils	Tanks no hyacinth	Tanks with hyacinth	Ponds	Swamps	Borrowpits, small	Borrowpits, long	Ditches	Rice-fields	Jute-fields	Pools, temporary	Pools, permanent	Drainage channels	Wells	Total
Darjeeling	6									4	2	2				2	9				15
Jalpaiguri											1				1		2				4
Dinajpur						21		1	1								20				5
Rangpur						2				27	2	21	1	2	6		4				48
Malda						27		43	1												5
Bogra																					58
Pabna																					74
Murshidabad										2	3	16		36	9		8	1			60
Nadia						8	2	1			5			2	8		13				31
Jessore														4			14				37
Khulna	7								1				1			2	25				32
24-Parganas																					10
Birbhum	1	5					2	16	2	3	1	2	2	10	3		5	16			60
Burdwan										25			9	12							54
Hooghly								1													1
Howrah		6																			16
Midnapur		2																			2
Bankura																					
Barisal									14			35		33	15		59				171
Faridpur	6							15				2									18
Dacca								16	1				1	12			29				21
Mymensingh								1	1				2	4							36
Chittagong				3								7	1	28			5				59
Noakhali								15	2												2
Tippura								2	2												2
	13	20		3		58	4	113	25	61	14	85	19	150	42	4	193	17			821

TABLE XIV

Districts	Streams	Rivers	Tanks no hyacinth	Swamps	BORROWPITS		Rice-fields	Pools		Total
					Small	Long		Temporary	Permanent	
<i>A culicifacies</i>										
Talpaiguri	2					1				3
Malda						1				1
Murshidabad		7		1						8
Birbhum		7							41	48
Burdwan	17		12	3	2	11		114	1	160
	19	14	12	4	4	11		114	42	
<i>A kochi</i>										
Darjeeling								1		1
Dacca						1	3			1
						1		1		
<i>A ludlowi.</i>										
Barisal								5		5
Chittagong							1			1
							1	5		228

## TABLE IX

*A. vagus*

Districts	Nullahs	Khals	Drams, kutcha.	Drams, pucca.	Streams	Irrigation canals	Dead rivers	Bhils	Tanks no hyacinth	Tanks with hyacinth	Ponds	Swamps	Borrowpits, small	Borrowpits, long	Ditches	Rice-fields	Jute-fields	Pools, temporary	Pools, permanent	Drainage channels	Wells	Total	
Darjeeling							6		18		98	3	15		5	2				.		5	
Jalpaiguri												4					4					137	
Dinajpur							1		8					2								13	
Rangpur									19					9			1	2				3	
Malda																						18	
Bogra					12	1						8	47					36	1			52	
Pabna																						95	
Murshidabad				10					1													53	
Nadia	12		21			2	14	5	4	5		3	59		14	10		11		6		138	
Jessore	1		10						2									18				25	
Khulna														13	6		5		4			26	
24-Parganas														3								3	
Birbhum				2																		2	
Burdwan	8			2	1				8	12	12	16	20	14	11	2		21	25			152	
Hooghly															4			8				12	
Howrah																						23	
Midnapur			10													5		2				5	
Bankura			5																			6	
Barisal																						13	
Faridpur																						15	
Dacca	6				4		8		1	2		2	16	1	4	3		6	7			59	
Mymensingh							5					6		1				8	18			22	
Chittagong																		11					
Noakhali			18		1				1				10	8	9			1				48	
Tippura	9				78								1			3		21	9			1	
																						121	
		36	64	14	96	3	46	5	62	19	118	46	169	52	55	25	5	151	69			6	1,041



*A. rossivagus*

Districts	Nullahs	Khals	Drams, kutcha	Drams, pucca	Streams	Irrigation canals	Dead rivers	Bhils	Tanks no hyacinth	Tanks with hyacinth	Ponds	Swamps	Borrowpits, small	Borrowpits, long	Ditches	Rice-fields	Jute-fields	Pools, temporary	Pools, permanent	Drainage channels	Wells	Total
Darjeeling											11											11
Jalpaiguri																						1
Dinajpur							1															
Rangpur																						
Malda																						
Bogra																						
Pabna																						
Murshidabad																						
Nadia		1									6			2	1				1			11
Jessore																						
Khulna															1							1
24 Parganas																						
Birbhum																						
Burdwan					1							9					4	2	1			3
Hooghly																						15
Howrah																						
Midnapur																						
Bankura																						
Barisal																						
Faridpur																						
Dacca													1						5			1
Mymensingh																						
Chittagong																						
Tippera					9								1		1			3				2
Noakhali																						13
		* 1			10		3				17	9	2	2	3	4		6	7			64

TABLE XVIII

Districts	<i>finestus</i>	<i>sinensis</i>	<i>barbirostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>rossi-vagus</i>	<i>acutus</i>	<i>paludus</i>	<i>culicifacies</i>	<i>tesselatus</i>	TOTAL
NULLAHS												
Birbhum	1			1								2
	1			1								2
KHALS												
Rangpur	43		6									49
Nadia	13			30	12	2	1	1				59
Jessore	11			13	1				1			26
Burdwan			1	11	8	2						22
Faridpur	6			2				6				14
Dacca	94		6	52	6			3				161
Mymensingh	6			4								10
Chittagong	4											4
Tippera	9			7	9			20				45
	186		13	119	36	4	1	30	1			390
STREAMS												
Jalpaiguri	5			10						2		17
Dinaipur	4											4
Rangpur	14			11								25
Bogra	12			30	12							54
Murshidabad	6					4						10
Birbhum	1			6		1	1					9
Burdwan					1					17		18
Mymensingh	6			5	4	1						16
Chittagong	2		3		1						1	7
Tippera	7				78	3	9	3				100
		57	3	62	96	9	10	3		19	1	260

TABLE XIII

Districts	Nullahs	Drains, kutcha	Dead rivers	Tanks no hyacinth	Ponds	BORROWPITS		Pools, temporary	Total
						Small	Long		
<i>A. maculatus.</i>									
Darjeeling								1	1
Jalpaiguri					2				2
Rangpur			1						1
			1		2			1	
<i>A. funestus</i>									
Jalpaiguri			2						2
24-Parganas		3		2	3				8
Birbhum	1								1
Hooghly			1						1
Chittagong			..				1	1	2
	1	3	3	2	3	1	1		18



TABLE XX

Districts	<i>sinensis</i>	<i>barbirostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>rossi-vagus</i>	<i>acutus</i>	<i>maculatus</i>	<i>culicifacies</i>	<i>koehn</i>	<i>indigenus</i>	<i>jamesi</i>	<i>jamesi x</i>	<i>maculipalpis</i>	TOTAL.
POOLS, TEMPORARY															
Darjeeling	10	2	4		9					1					33
Dinajpur	32	9	2												36
Rangpur	44	20	16												80
Malda				2	5										7
Bogra	11			1											12
Pabna	4	4	16	36	1										61
Murshidabad		8		11	2	1									21
Nadia	35	13	12	18	6										85
Jessore	43	14	8												65
Khulna	66	25	15	5				1					4		116
Birbhum	1		3												4
Burdwan	24		31	21		2			114			1		2	233
Hooghly	12	5	4	8	38										29
Midnapur	11		9	2											22
Barisal	16	59	6	6			6				5				98
Dacca	32		19	8											59
Mymensingh	12	29	1	11											53
Chittagong	34	5	12	1											52
Tippera				21		3									24
	387	193	158	151	61	6	6	1	114	1	5	1	4	2	1,090
POOLS, PERMANENT															
Malda	21		6		3		2								32
Murshidabad		1	15												16
Nadia			9	6											15
Jessore	10			4											14
Birbhum					8	1			41						50
Burdwan	49	16	62	25	14				1						167
Faridpur				7	4	1									18
Dacca	6		8	18		5									31
Tippera				9											9
	86	17	100	69	29	7	2		42						352



TABLE XXII (contd )

Districts	<i>sinensis</i>	<i>barbirostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>rossi-vagus</i>	<i>acoutus</i>	<i>indicus</i>	<i>tesselatus</i>	TOTAL
JUTE-FIELDS										
Darjeeling	4	2			1					7
Dinajpur	6			4						10
Malda	1			1	2					4
24-Parganas	5	2			1					8
	16	4		5	4					29

TABLE XXIII

## DITCHES

Districts	<i>sinensis</i>	<i>barbirostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>rossi-vagus</i>	<i>acoutus</i>	TOTAL
Dinajpur	22	1	3	5	5			36
Malda	6	2		2	4			14
Bogra	38	6						44
Pabna	4		10					14
Murshidabad	24	36	5					65
Nadia				14		1		15
Jessore	9	2						11
Khulna	9	4		6			1	20
24 Parganas						1		1
Birbhum								..
Burdwan		10	10	11	8			39
Hooghly		12		4				16
Barisal	29	33	10	4			4	80
Dacca	32	12	19				7	70
Mymensingh	36	4	3					43
Chittagong	25	28		9		1	2	65
	234	150	60	55	17	3	14	533

TABLE XVII  
DYING RIVERS

Districts	<i>sinensis</i>	<i>barburostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossi-vagus</i>	<i>acutus</i>	<i>funestus</i>	<i>maculatus</i>	<i>culicifacies</i>	<i>jamesi</i>	<i>pallidus</i>	<i>jamesi</i> x	TOTAL
Jalpaiguri	21		24	6				2					.	53
Rangpur	8	21	8	1		1			1				.	40
Malda	15		1									.	1	17
Bogra	23	2	6				4							35
Pabna		27	73	12	5						2			
Murshidabad			1							7				8
Nadia	4		25											29
Jessore	29	8	72	14										123
24-Parganas												.		-
Birchum					4	1		1		7				13
Burdwan					.								.	-
Hooghly	22						38							60
Midnapur					..								.	
Faridpur	1		38	8					.					47
Dacca	1		1	5								.		7
Tippera	2					1	16				.			19
	126	58	247	46	9	3	58	3	1	14	2		1	570

TABLE XXV  
LONG CUTTINGS

Districts	<i>sinensis</i>	<i>barburostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossi-vagus</i>	<i>acomtus</i>	<i>funestus</i>	<i>culicifacies</i>	<i>kochi</i>	<i>jamesi</i>	<i>pallidus</i>	<i>jamesi</i> #	<i>stephensi</i>	TOTAL.
Jalpaiguri	32		17								2				51
Dinajpur	4		54	2											60
Rangpur	49	1	42	9											101
Bogra	10		10												20
Pabna															
Murshidabad			4												4
Nadia	30		6			2									38
Jessore	15		11	1											27
Khulna	4	2	2	13	7										28
24-Parganas	1	1		3	9										14
Birbhum	4		33									1			38
Burdwan	42	2	80	14	6		4		11			1	42	1	203
Hooghly	59	9	19									1			88
Faridpur	51										2		4		57
Dacca	65	1	23	1			7			1					98
Mymensingh	86	2	17	1											106
Chittagong	11	1	7	8			8	1							36
Noakhali											5		37		42
Tippera	8		8				12								28
	471	19	333	52	22	2	31	1	11	1	9	3	83	1	1,039

TABLE XXVI  
BHILS

Districts	<i>sinensis</i>	<i>barburostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossi-vagus</i>	<i>acomtus</i>	<i>jamesi</i>	<i>pallidus</i>	<i>jamesi</i> #	<i>maculipalpis</i>	TOTAL
Malda	10											10
Bogra	30		8									38
Nadia	29	2	40	5			1	3		2		82
Jessore	43		47				2		1			93
Birbhum	9		14								1	24
Burdwan	38	2	11						2			53
Hooghly			4									4
Faridpur	22		8									30
	181	4	132	5			3	3	3	2	1	334

TABLE XIX

Districts	<i>sinensis</i>	<i>barburostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>acomitus</i>	<i>funestus</i>	TOTAL
DRAINS, KUTCHA								
Murshidabad				21	25			46
Nadia				10	4			14
24-Parganas	14	7			1	1	3	26
Burdwan	7	5			4			16
Midnapur		6	4	10	7			27
Bankura	10	2		5				17
Barisal	5							5
Chittagong				18				18
	36	20	4	64	41	1	3	169
DRAINS, PUCCA								
Murshidabad			5	10	6			21
Birbhum				2	..			2
Burdwan			..	2				2
			5	14	6			25
IRRIGATION CANALS								
Bogra				1				1
Nadia	2			2				4
Hooghly	6		2					8
Chittagong			1					1
	8		3	3				14

TABLE XXVIII  
TANKS WITH HYACINTH

Districts	<i>sinensis</i>	<i>barbistris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>acutus</i>	<i>jamesi</i>	<i>jamesi</i> *	<i>pallidus</i>	Total
Jalpaiguri	32		.					.		32
Malda	8	1	1							10
Bogra	15	1	8		.					24
Nadia	6							1		7
Jessore	20		10	5		—		1	.	36
Khulna	10	1								11
24-Parganas	2			.		1				2
Birbhum	16		11		4				1	32
Burdwan *	26	2	42	12					.	82
Hooghly	1		6							7
Barisal	7	14								21
Dacca	36	1	3	2		12				54
Mymensingh	13	1	7							21
Chittagong	68		88			2	7			165
Noakhali	45	2	.			26				73
Tippera	60	2	2			31				95
	365	25	178	19	4	71	7	2	1	672

TABLE XXI

Districts	<i>sinensis</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>stephensi</i>	TOTAL
DRAINAGE CHANNELS						
Hooghly	4	12				16
WELLS						
Nadia				4	1	5
Midnapur	4		6			10
	4		6	4	1	15

TABLE XXII

Districts	<i>sinensis</i>	<i>barbirostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossii-vagus</i>	<i>aconitius</i>	<i>indlowi</i>	<i>tesselatus</i>	TOTAL
RICE-FIELDS										
Darjeeling	13			2						15
Dinajpur	17	1	2							20
Malda	5									5
Bogra	5									5
Murshidabad	12	9		10						31
Jessore	12	8	3					..		23
Burdwan	16		4	2		4				26
Hooghly	3	3								6
Midnapur	6	6		5	8					25
Barisal	23	15	4	3			1			46
Dacca	17									17
Mymensingh	25		2							27
Chittagong	24		8				1	1	2	36
Tippera	11		24	3			9			47
	189	42	47	25	8	4	11	1	2	329



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TABLE XXIV  
BORROWPITS, SMALL

Districts	<i>sinensis</i>	<i>barbirostris</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossi</i>	<i>rossi-vagus</i>	<i>acutus</i>	<i>funestus</i>	<i>culicifacies</i>	<i>jamesii</i>	<i>pallidus</i>	<i>jamesii</i>	Total
Darjeeling	13	2	4		5								24
Jalpaiguri	3		25	15					1	1			45
Dinajpur			13										13
Malda	3								1				4
Bogra	6	21											27
Pabna				47									47
Nadia	20	16	3	59	9						1		108
Burdwan	35	2	3	20	7				2			2	71
Hooghly			23										23
Barisal	25	35	15										75
Faridpur	23	2					6						31
Dacca	38		6	16		1	7						68
Mymensingh	29									2		7	38
Chittagong	29	7	25	10		1		1					73
Noakhali	8		8	1			12						29
Tippera				1									1
	232	85	125	160	21	2	25	1	4	3	1	9	677

The fixed valleys of the great rivers are of very variable width, generally bounded by steep high banks, they are called Khadar, the adjoining high land being known as Bhangar. The deep, low-water, channel of the river oscillates within the Khadar, or river-plain, the whole of this being liable to inundation from the floods, and to constant erosion and re-formation by the action of the current. Khadar-mati is very nearly the native equivalent for "alluvial land." But though there is always a large total area of true alluvial land in the Khadars of the great rivers, it is possible that, on the whole, these Khadars are undergoing denudation, that the river-bed is deepening, and that the new alluvial land formed by its changes of position may be progressively lower than the older patches removed by the same process. It has not yet been defined how much, or if any portion, of the eastern districts come within the sub-deltaic conditions that prevail in the Lower Provinces, where the river-action is broadly formative. The whole of the province of Oudh would come under one or other of these descriptive terms—Bhangar or Khadar land.

Whether the great rivers are raising or lowering their Khadars. The question whether the great rivers have at present a tendency to deepen or to raise their channels is one of much importance in relation to engineering works, and of great interest to the geologist. Within deltaic regions, where the rivers are essentially formative, the process is sufficiently understood, the bed and banks of the main channel are raised, till the contract of level determines a gradual set of the water to lower ground, through some minor distributary, the new channel is at first scoured out to the capacity of the main channel, when the raising action recommences. Within the narrow river-plain of the Khadar, there might be no general feature to betray which process is in force. The river would oscillate pretty much alike in either case, removing and replacing the patches of alluvial land. Still it seems likely that careful enquiry among the natives cultivating the Khadars would elicit some grounds for judgment, as, if any very old patches of alluvium were no longer inundated by the highest flood, one might infer that the river had lowered its channel. The *a priori* conditions may be stated briefly thus: whether a river is cutting or depositing, depends of course, upon its velocity and upon the charge of solid matter, wholly or partially suspended in it. As regards the first condition, it can be broadly stated that the slope (and hence the velocity) within the Khadars, is everywhere much above that at which silt-carrying rivers become on a large scale depositing rivers, at Kanhpur the fall is nineteen inches per mile, at Allahabad thirteen, while in the sub-deltaic region at Patna and Rajmahal it is only six inches, and in the Delta proper it lowers to three inches. It is moreover certain that for eight or nine months of the year, the great rivers rush from their gorges in the mountains as torrents of clear water, or only, in the hot months, discoloured by fine glacial mud, immediately upon entering the Khadar, however, the water becomes more or less charged with silt and continues so throughout its course. For these months then the river must be denuding its channel. During the flood season, on the contrary, the water issuing from the mountains is highly charged with detritus, which is, to some extent at least, gradually deposited as the slope of the channel becomes lowered in the Khadar. It would be difficult to conjecture to what distances within the plains coarse shingle and gravel might be rolled along by the scour of the current in extreme floods during successive seasons. Large stones not

TABLE XXVII  
TANKS WITHOUT HYACINTH

Districts	<i>swiensis</i>	<i>barbrostis</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossi-vagus</i>	<i>acanthus</i>	<i>finestus</i>	<i>maculatus</i>	<i>ciliatifacies</i>	<i>koehi</i>	<i>indlowi</i>	<i>lesseleatus</i>	<i>jamesi</i>	<i>pallidus</i>	<i>jamesi x</i>	<i>maculipalpis</i>	<i>stephensi</i>	Total
Jalpaiguri	20		61	18	3														102
Dinajpur	20																		20
Rangpur	19	1	2	8															22
Malda	6																		14
Bogra	35	43	29	19										1					84
Pabna																			43
Murshidabad	14	1	46	1	2														64
Nadia				4	4														8
Jessore																			14
Khulna			12	2		..		2											2
24-Parganas																			14
Birbhum	11		29	8						12					4				44
Burdwan	36	16	29				9												110
Hooghly	47		2				2												51
Howrah	34	1	3																38
Midnapur	4	4	10																18
Barisal	13	15	17																45
Faridpur	18	16	54												1				89
Dacca	25	1	24	1	1		9							1					62
Mymensingh	11																		11
Chittagong		15																	16
Noakhali	10		10	1			12												32
Tippera	23		33																56
	346	113	361	62	10		32	2		12				2	5	.			945

The phenomena under notice have been only incidentally examined, so that the sketch here given is very incomplete and open to correction

*Age of the Bhangar land*—It having been shown that the great mass of the plains-deposits belongs to a bygone period of formation, it develops upon the geologist to ascertain the age and nature of the process. Very little progress has as yet been made to that end, the systematic study of the question not having been taken up. Some have maintained that the deposits are marine or estuarine, others, as seems most likely, that they are, at least to any observed depth, purely fluvial, by a process like what is now going on in the Bengal Provinces. No trace of marine organisms has been found in them. But some bones of terrestrial mammalia were got in a hard bed of calcareous gravel in the bed of the Jamna near Etawa, and which seem to belong to species or varieties now extinct, so that those deposits will probably take rank among the later Tertiaries. From observations made in sinking wells along the line of railway, one of the engineers has stated the general section of the Ganges-Jamna Doab south of Aligarh to be—loam 35 feet, blue silt 30 feet, strong clay 20 feet, resting on a water-bed of reddish sand, from which the water rises some 30 feet. The bed of clay slopes from north to south at about two feet in the mile, the surface sloping about eighteen inches in the mile. The water obtained from the blue silt is always more or less saline. The only deep section of the plains-deposits is from the boring for an artesian well at Ambala. This position, a little to the west of the Ganges-Indus water-shed, is on the zone of recent deposits, the river channels are all superficial, and become lost in the desert country to the south. There is nothing in the section of the boring to mark a change from these surface deposits to others of an older period. None could, indeed, have been expected, as it is only on an extended horizontal section that a plain of denudation, such as that of the present Ganges-Jamna Doab, could be detected between any older beds and perfectly similar material recently overlaid upon them. There is moreover no presumption that any such break exists in the plains-deposits west of the main water-shed, or at least at that water-shed. A single boring, too, can tell little or nothing of the arrangement of the strata. The depth reached was 455 feet, or 450 feet above the sea-level. Frequent alterations of clay and sand were passed through. At 286 to 296 and 400 feet to 417 feet coarse gravel and large stones were found, strong beds of clay occurring again beneath.

TABLE XXIX

Districts	<i>sinensis</i>	<i>barbivrosus</i>	<i>fuliginosus</i>	<i>vagus</i>	<i>rossii</i>	<i>rossi-vagus</i>	<i>aconitus</i>	<i>funestus</i>	<i>maculatus</i>	<i>culicifacies</i>	TOTAL
PONDS											
Jalpaiguri		4	41	98	38	11			2		194
Malda	2			1	4						7
Bogra	6	27		2	1						36
Murshidabad	6	2	6								14
Nadia	2			5	6	6					19
24-Parganas	6		2		3			3			14
Burdwan	5	3	4	12	3						27
Hooghly	2	25									27
	29	61	53	118	55	17		3	2		338
SWAMPS											
Darjeeling	2	2	3	3							10
Jalpaiguri	25		2								27
Dinajpur	12	1		4							17
Rangpur	42		2								44
Malda	11	2	12	4							29
Bogra	65		47	8							120
Pabna	12		12								24
Murshidabad	6	3			8					1	18
Nadia			8	3							11
Jessore	10	5	5								20
24-Parganas	4						1				5
Burdwan	58	1	83	16	18	9	5			3	193
Hooghly	15										15
Midnapur	10										10
Faridpur	23		5								28
Dacca	140		59	2							201
Mymensingh	26		2	6							34
Tippera	89		22				23				134
	550	14	262	46	26	9	29			4	940

The least resistant group either does not exist at all or is hæmolysed at once. This observer is of opinion that the spleen filters off from the general circulation, the less resistant cells and that the increased fragility is not due to the sojourn of the cells in that organ. That the blood detained in the spleen may undergo other changes is indicated by the observations of Cruickshank (1926) that blood from the splenic pulp is more concentrated in hæmoglobin than the blood of the body, and by those of Brann and Bischoff (1926) that splenectomy increases the resistance of hæmoglobin to destruction by NaOH. The influence of the spleen on the red blood corpuscles is admirably discussed by Krumbhaar (1926) in his review of the functions of that organ.

The observations upon which this paper is founded were made in the Mayo Hospital, Lahore, on a series of cases some of whom showed enlargement of the spleen and liver or enlargement of the spleen only, while others had cirrhotic livers with or without splenomegaly. One group gradually merged into the other so that it is probable that both conditions resulted from different degrees of the same pathological process. All the patients had a history of chronic malaria with irregular bouts of fever, and coming from the Punjab were presumably infected from childhood. The non-cirrhotic cases were all more or less anæmic, the anæmia being of a secondary type with leucopœnia, and showed evidence of increased blood destruction by the presence of urobilinuria and hyperbilirubinæmia. Many of those admitted during the fever season (October and November) suffered from irregular pyrexia in hospital, but no malarial parasites were found in the peripheral blood. None were jaundiced and none had hæmorrhages. Schistosomiasis, the supposed cause of Egyptian splenomegaly (Manson-Bahr, 1925), was not present as this infection does not exist in the Punjab. Toxic jaundice of the type seen in malaria subjects in bad 'fever' seasons, as described in a previous communication (Hughes, 1926), attacks these patients, but whether this jaundice is the result of a superadded disease or is due to an exacerbation of the chronic condition is not known.

The degree of portal obstruction, as indicated by ascites and the development of the collateral venous circulation, varied in the cirrhotic group. In some, (the worst), cases, the obstruction seemed to be almost complete, while in others there was only a slow accumulation of fluid in the abdomen with little enlargement of the collateral channels. In cases of the latter type, however, which could be observed for any length of time, a gradual increase of the cirrhosis was seen. Patients without ascites sought admission to hospital either on account of fever or because of the mechanical inconvenience caused by an enlarged spleen. Individuals of the class to which these patients belonged do not, as a rule, come into hospital unless they are seriously ill or realise that their complaints cannot be benefited by the indigenous methods of treatment to which they resort, so that a large proportion never come under observation until cirrhosis is definitely developed. For this reason, early and transition cases are comparatively rarely seen in the wards. Cirrhotic cases run a progressively downward course and die in a few months,

## APPENDIX

Speaking of the Gangetic plains in the North-West Provinces, Medlicott says —

‘The geology of the North-West Provinces is conveniently separable into three divisions, corresponding to three district geographical regions. Twenty-three of the thirty-five revenue districts are entirely on the Gangetic plains. Three districts on the North, Dehra Dun, Garhwal, and Kumaon belong altogether to the Himalayan region. Out of nine districts on the south, seven are in very large proportion covered by the plains-deposits, three only being in whole or in great part within the rock-area of the Peninsula of Hindustan. It is at once apparent that these geographical divisions are also strictly geological, and it may be here stated that no identification has as yet been made out between the rocks within these provinces on the north and on the south of the plains. The formations of these several regions may be noticed in the following order —

### I THE PLAINS

Terms applicable to these deposits—The middle region naturally claims first attention. It is often spoken of as “the alluvial plains of the Ganges,” or by such-like expressions. In a general sense, these terms are admissible, there is no doubt that the materials forming the plains were contributed by the Ganges and by its tributaries. But in this range of meaning, the Sivalik-déposits might claim to be included for it has been shown that their materials, too, were conveyed through the existing Himalayan drainage system. On the other hand, by confirming the word “alluvium” to its strict geological meaning—to ground subject to flooding from the very channels that now exist—the alluvial ground of the North-West Provinces becomes comparatively small. It is necessary to specify still further to bring out the distinction to be made in the area under notice, the word “alluvium” is scarcely understood unless as applied to fine-deposits from tranquil inundation, and it applies to such indiscriminately, whereas from the proper geological point of view, the distinction to be indicated is what ground is undergoing increase from whatever form of deposition, and, on what ground abrasion (denudation) is in permanent action, or, in other words, where river action is formative and where it is destructive.

*Bhangar and Khandar lands*—A large proportion of the plains area in these provinces is permanently undergoing denudation. The main rivers run through it in confined and fixed valleys, the flood-level of the waters being well below the general level of the country. Several considerable streams, as the Hindan, take their rise within this area, and though subject to local overflow, with deposition of alluvium, they must, on the whole, carry away annually a large quantity of earth.



TABLE II.

*cirrhosis of the liver.*

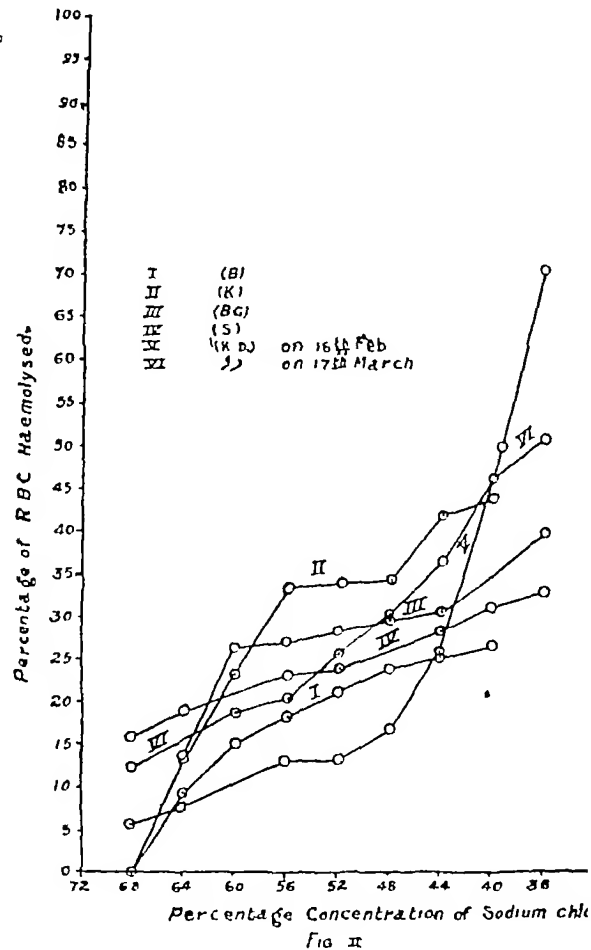
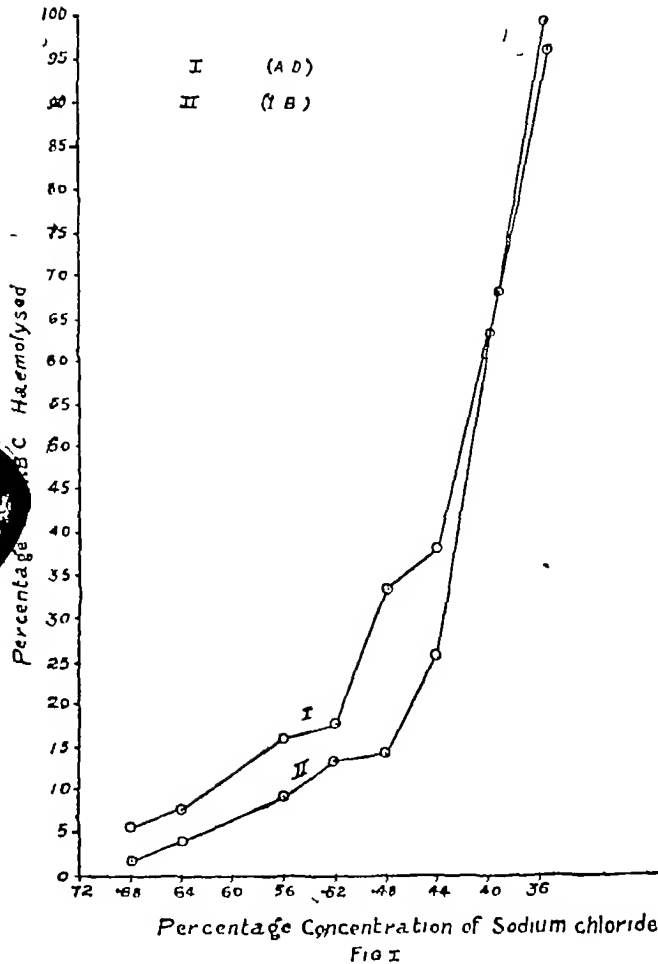
um- f C	Percentage of R. B C hæmolysed in the following percentage concentrations of NaCl									REMARKS
	0 68	0 64	0 60	0 56	0 52	0 48	0 44	0 40	0 36	
00		9 5	15 2	18 2	21 1	23 5	25 4	26 5		Increased corpus- cular resistance. The patient died a few days later
1—	3 7	5 9		12 5	15 7	24 0	35 9	63 5	98 2)	
00		13 8	23 3	33 4	33 8	34 1	41 8	44 0		Increased resist- ance
00		13 1	26 1	27 1	28 3	29 9	30 5		39 8	Do
00	15 9	19 0		23 2	23 7		28 5	31 1	32 9	Do
00	2 3	12 1		20 5	44 4	89 9	100	100	100	Decreased resist- ance Glucose tolerance test on 24-11-26, B S percentage be- fore and at interval of $\frac{1}{2}$ , 1, 1 $\frac{1}{2}$ and 2 hours after ingestion of 50 grms glu- cose 177, .246, 324, .246, .232
00	11 4	19 6		27 7	37 5	61 4	78 2	97 3	100	Decreased resist- ance.
00	0 43	4 5		23 0	27 5		96 6	100	100	Do Glucose tolerance test on 18-12-26, Blood Sugar 146, 177, 216, .202, .221
00	5 7			12 9	16 3	98 3	100	100	100	Decreased resist- ance
00	8 3	10 5		14 4	33 5	46 1	46 4	96 3	98 5	Slightly decreased resistance in cer- tain concentra- tions but on the whole the fragil- ity is normal
00	4 3	11 7		33 9	42 9	44 3	44 5	84 2	96 0	Almost normal
00	0 6			0 6	23 7	26 2	36 6	74 2	97 5	Resistance increas- ed at higher con- centrations of NaCl

early and transition cases are comparatively rarely seen in the warus cir-  
rhotic cases run a progressively downward course and die in a few months,

being found in the bed of the river in the dry season may not be a safe indication of the case, as it is conceivable that they should always be buried under lighter deposits as the flood subsided. Whether or not the rivers are able, even with the assistance of the clear water for eight months of the year, to carry out of the Khadar all that they carry into it in the flood season, cannot be determined without careful observation, but from all the considerations mentioned, it would seem likely that throughout the greater part of the Khadar, the balance is in favour of erosion. Any tendency of the gorges ought to be discoverable from the effect on the canal-heads at Hardwar and Fyzabad. Continued observations on the silt in the water, uniformly conducted at distant places, as at Fatehgarh, Kanhpur, and Allahabad, might indicate whether erosion or deposition is taking place within the Khadar region. But the most satisfactory test would be, continued registration of the rise and fall of the water on permanently fixed gauges, to be checked by an annual exact measurement of the low-water river-section at each gauge.

*Bhabar and Terai land*—Independently of such tracts on the eastern borders of the province as come within the sub-deltaic region of the great rivers, there is a considerable stretch of country where the drainage is formative. The minor streams from the outer skirts of the mountains do not run on into the plains in deep channels cut through deposits of earlier times, they flow, at least for many miles in broad shallow and ever-shifting beds formed of materials brought down by themselves. The load of shingle, gravel, sand and earth washed into these torrents by the heavy rainfall from the precipitous slopes of the Sivalik hills, formed of soft conglomerates, sandstones and clays, is far more than the current can carry into the main rivers. It is possible too, as has just been discussed, that something of the same kind takes place in the upper reaches of these rivers themselves. There is thus, along the northern margin of the plains, a broad belt of ground, the formation of which is strictly "recent". The portion of it next the hills having a steeper slope than the rest, is chiefly composed of shingle and gravel with a filling in of sand and earth. This is the forest-bearing zone known as the Bhabar. Except in the rainy season, it is devoid of water, streams of considerable volume soon sinking into the porous ground to reappear (at least in part) along the lower fringe of the coarse deposits. This second zone, though having, on the whole, a considerable slope, greater than the general slope of the plains, is thus made watery and swampy, it is well known as the Terai. West of the Ganges this formative process is specially active owing to the greater development here of the soft Upper Sivalik rocks, which are the most abundant source of detritus. Some years ago, excavations in connection with the Eastern Jamna Canal, brought to light the ruins of an ancient town. The Terai in the Jamna-Ganges Doab is scarcely a noticeable feature, owing probably to the good natural drainage, the watershed being here 400 feet above the Ganges at Hardwar. Eastwards from the Ganges, the Terai becomes more and more distinct. In the same direction, remnants of an ancient Bhabar deposit become frequent and of increasing elevation, till in the far east at the base of the Sikkim-Himalaya, they stand at 1,000 feet over the actual torrents. To the south of the plains, some analogous cases of recent deposits may be found, but they are altogether insignificant, the larger rivers there also running in channels which they do not overflow to any extent.

however, such as appears in Orahovatz's curves. In other words, the distinction between average grown and new cells is not evident. In Figure IV are four curves indicating increased fragility of the erythrocytes, two from patients with splenomegaly but without cirrhosis, and two from cirrhotic cases. Increased resistance to saline haemolysis in cases from both groups is illustrated in Figure III and Figure II respectively. It is to be noted that on the whole, low red cell counts are associated with increased corpuscular fragility, while in some patients with increased resistance, the red blood count



is above normal (*vide* appendix). Individuals with increase of corpuscular stability had, as a rule, suffered longest from the disease, and a marked increase of resistance with cirrhosis was found only in severe cases. Patient No 1, Table II, died a few days after the observation recorded was made. An increase of resistance with the progress of the disease is seen in patient No 13, Table II, and to a less marked degree in patients Nos 10 and 11, Table II. In some cases normal or nearly normal curves were obtained. This may be due to the time of blood examination having coincided with the period of transition from increased fragility to increased resistance. The

# OBSERVATIONS ON TROPICAL CIRRHOSIS OF THE LIVER WITH SPECIAL REFERENCE TO THE FRAGILITY OF THE RED BLOOD CELLS

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CLINICAL and experimental data furnish evidence of the influence of the spleen and liver on the fragility of the red blood cells in hypotonic saline solution. In acholuric jaundice the corpuscles are always pathologically fragile, and this fragility has been found to disappear, in part at least, after splenectomy. In obstructive jaundice and in splenic anæmia (Banti's disease) there is an increased resistance to saline hæmolysis (Beaumont and Dodds, 1926), and a similar condition results from removal of the spleen in normal animals (Krumbhaar and Muller, 1923). Orahovats (1926) has plotted the fragility curves of the red blood cells from the general circulation and from the spleen pulp of normal cats, and has found that the latter are less resistant to hypotonic salt solution than the former. Further, the curve of the spleen blood differs in shape from that of blood from the general circulation. Orahovats confirming Brinkman (1921-1922) has demonstrated that in the circulating blood there are three distinct groups of cells with different resistances to hypotonic saline solution. The first and most resistant group forms about 10 per cent of all cells, the group with a middle resistance 80 per cent and the third group with very little resistance 10 per cent. Brinkman considers the first group to consist of young cells, the third of old, and the middle group of average grown cells. In blood from the splenic pulp Orahovats has found that the second and third groups could not be distinguished from each other.

'splenomegalic cirrhosis with icterus' the histological picture of Banti's disease was found only twice, viz, in two persons who had lived in malarious countries. A feature of the histological changes described is the proliferation of the mesenchymal elements of the spleen and liver belonging to the reticulo-endothelial system. This system is represented in the spleen by the reticulum cells of the pulp, the endothelial cells of the sinuses and the splenocytes (Aschoff, 1924). It is well known that the cells of this system take up from the circulation particulate matter, such as India ink or carmine, and foreign colloids, such as colloidal silica, and that repeated injections of such substances causes them to enlarge and proliferate (Gye and Purdy, 1922, 1924). McNee (1913) showed that this phenomenon was also brought about by destruction of red blood corpuscles. Here the cause is the elaboration of an excessive amount of bilirubin from the liberated hæmoglobin, the preparation of bile pigment being a normal function of these cells. While the reticulo-endothelial proliferation seen in the sections is probably connected with blood destruction in the early stages, it cannot be definitely said whether this proliferation is entirely caused by increased breakdown of the red blood corpuscles or whether it is the result of some other stimulus. That the changes in corpuscular fragility are at all events related to the spleen, and most probably to the reticulo-endothelial system as a whole, is suggested by the effects of splenectomy in hæmolytic jaundice and in normal animals.

In the absence of more detailed histories and observations during the progress of the disease, and of more post-mortem examinations, it is not possible to assess the exact rôle played by malaria in this condition. Although no parasites were seen in the sections or in the blood of any of the patients, one cannot conclude that malaria was absent in all cases. In chronic sub-tertian infections the parasites live in the internal organs, and, as Manson-Bahr (1920) has pointed out, it may be very difficult to demonstrate them in these cases. It is noteworthy that all the patients examined came from the lower rural classes amongst whom the treatment of malaria would be defective, partly, at all events, because of the indifference with which an ordinary attack of fever is regarded by these people. It is possible that the hypertrophy and multiplication of the reticulo-endothelial cells is, to some extent, an expression of immunity to repeated malarial infections. There is much evidence, quoted by Sacks (1926) in his comprehensive review, to show that these cells play an important part in general and local immunity processes. Splenectomy in mice, combined with injections of colloidal iron oxide to block the remainder of the reticulo-endothelial system, abolishes the power to produce hæmolysins (Bieling and Isaac, 1922). Blocking the reticulo-endothelial cells diminishes the response of tuberculous animals to tuberculin (Minami, 1925), and a combination of splenectomy with injections of sugar of iron and trypan blue causes suppression of tuberculin hypersensitivity (Freund, 1926). Rats immunised against streptococcus vaccine lose the power to store vital dyes (Paschkis, 1924) and rabbits immunised against pneumococci behave like non-immune animals after injection with India ink (Singer and Adler, 1924). Injection of manganese chloride, a substance

Case.	Date	Clinical notes	Enlarge- ment of the spleen in inches below the costal margin	Enlarge- ment of the liver in inches below the costal margin	Biliru- bin con- tent of the serum in Van den Bergh units	Initial n ber o R B
1 B, æt. 50 years	20-10-26	Duration of ascites, 5 months	Palpable		17	6,111,0
						(Norm.
2 K, æt. 8 years	21-10-26	Duration of ascites, 7 months	1½	Palpable	Normal	5,450,0
3 B G, æt. 30 years	26-10-26	Intermittent fever preceding the disease. Dura- tion of ascites, several months	3	3	0.42	5,050,0
4 S, æt. 25 years	29-10-26		2½		Normal	6,110,0
5 M B, æt. 45 years	23-11-26	Duration of ascites, 1½ months	4	1½	17	3,470,0
6 M, æt 40 years	16-12-26	Duration of ascites 3 months	4½	1	..	1,840,0
7 F, æt. 40 years	17-12-26	Duration of ascites 2 months	3	.	..	4,650,0
8 H, æt. 60 years	7-1-27	Duration of ascites, 2 months			..	4,060,0
9 Ma, æt 25 years	3-2-27	Duration of ascites, 4 months		1½	..	5,880,0
10 G, æt. 50 years	12-2-27	Duration of ascites, about 6 months			1.25	5,060,0
	23-2-27				10	3,550,0

When cirrhosis of the liver is established the outlook for these patients is hopeless. Symptoms are relieved by tapping the distended abdomen and by other measures, but death ensues in a few months from intercurrent infection, progressive weakness or hepatic insufficiency. In the earlier stages, especially when pyrexia is present, some cases are benefited by anti-malarial treatment. X-Rays have been found to cause reduction in the size of the spleen but not to produce any permanent improvement. In view of the good effects of splenectomy in certain cases of acholuric jaundice and thrombocytopæmic purpura and in Banti's disease, the advisability of removing the spleen in the pre-cirrhotic stage should be considered. The result would depend on the degree to which the liver is already affected. When malarial infection is present this should of course be vigorously treated.

### CONCLUSION

1 A condition beginning with splenomegaly and leading to enlargement and eventually cirrhosis of the liver is described as occurring in persons subject to repeated malarial infections.

2 In this early stage of the condition there is generally increased fragility of the red blood cells in hypotonic saline solutions.

3 In advanced cases this fragility disappears and there may be increased corpuscular resistance.

4 The relation of the condition to malaria is discussed.

Our thanks are due to Major V. Mirajkar, F.R.C.S., I.M.S., Professor of Physiology, King Edward Medical College, Lahore, for permission to work in the Physiological Laboratories, and to Drs. Mohammad Yusaf and Fakharud Din for assistance during the investigation.

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but owing to the fact that these patients seldom die in hospital no post-mortem examinations were obtained. During the observations one case did die in hospital from hepatic insufficiency with restlessness, delirium, convulsions and coma, but an autopsy was not permitted. Sections of spleen and liver from similar cases were, however, obtained through the courtesy of Lieut-Col H. M. Mackenzie, I.M.S., Professor of Pathology, King Edward Medical College. The sections were from patients showing the clinical picture of hepatic cirrhosis with splenomegaly, ascites, etc. In the spleen the microscopic changes observed were (1) an increase in thickness of the fibrous capsule and trabeculae, (2) great increase of fibrous tissue in the walls of all the blood vessels, (3) proliferation of the reticular, endothelial and pulp cells, (4) presence of bile pigment and hæmosiderin, and (5) more or less fibrosis of the Malpighian corpuscles and of the pulp. In some sections the lymphoid follicles were converted into masses of fibrous tissue and fibrous bands of appreciable thickness traversed the pulp. The liver sections showed degenerative changes in the glandular cells and multilobular cirrhosis, with a tendency for the fibrous tissue to invade the interior of the lobules. In all the specimens, the fibrous tissue was more or less cellular, the cells resembling the Kupfer cells. No malarial parasites were seen. The impression obtained from an examination of the sections was that the reticulo-endothelial elements of both spleen and liver first underwent proliferation, and that fibrous tissue formation occurred at a later period, especially in the liver. Some patients had albuminuria with occasional hyaline and granular casts, and when this was combined with œdema of the lower extremities, which is often seen in cirrhosis of the liver, the extent to which the kidneys contributed to the anasarca had to be assessed. If renal tests indicated any considerable impairment of kidney functions such cases were not included in the series.

### *Method*

The fragility of the red blood cells in hypotonic salt solutions was estimated by the method described by Orahovats (*loc cit*). After a preliminary red blood cell count dilutions of blood, 1 in 200, were made in hæmocytometer pipettes in solutions of NaCl ranging in strength from 68 per cent to 36 per cent. Counts were made after about 20 minutes and the percentage hæmolysis plotted against the strength of the salt solution. Blood was drawn directly into the pipettes. Estimations were also made of the bilirubin content of the blood serum. The serum in all cases gave the indirect Van den Bergh reaction.

### *Results*

(Table I gives the results obtained on patients without obvious hepatic cirrhosis and Table II the results on patients with definite portal obstruction.) Figure I shows two normal fragility curves. Hæmolysis is seen to begin in 68 per cent and to be almost complete in 36 per cent NaCl. The curve rises slowly at first, resembling in this respect the normal curve for cat's corpuscles as described by Orahovats. There is no slowing down at the end,





degree of splenomegaly in patients without cirrhosis is seen to be greater when the corpuscular fragility is increased than when it is normal or diminished

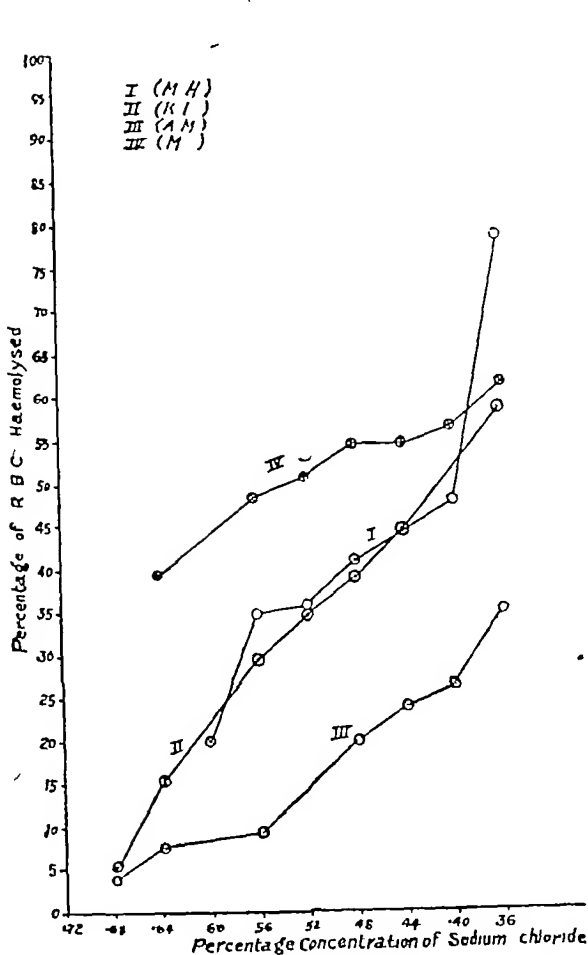


FIG III

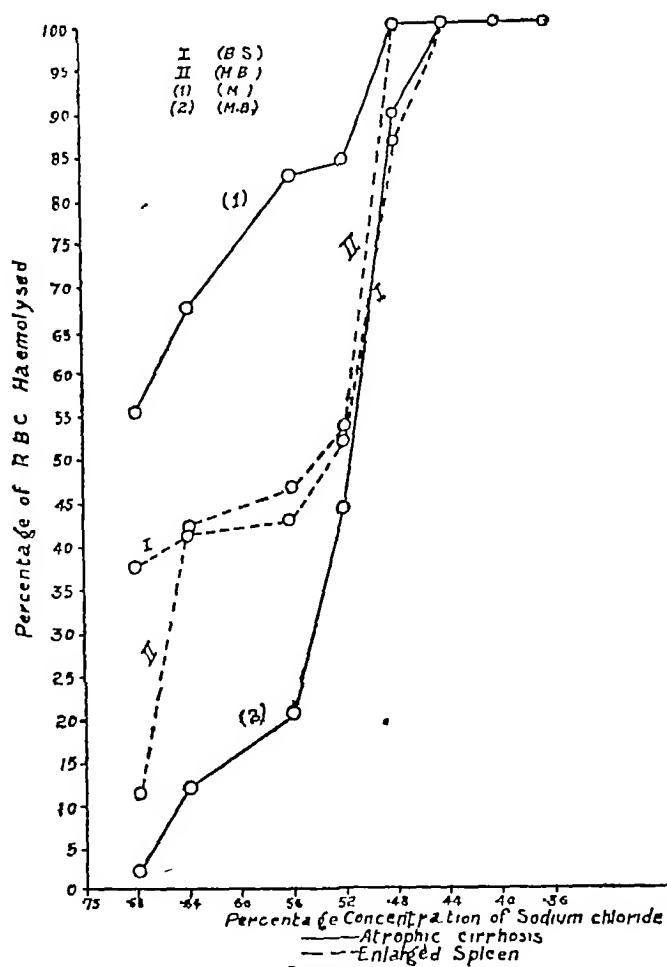


FIG IV

### Discussion

Although the clinical picture in advanced cases resembles that of so-called alcoholic cirrhosis alcohol cannot be reckoned a causative agent in this condition, as most of the individuals never tasted alcohol. In the early stages some patients with marked hæmolysis resembled mild cases of acholuric jaundice. This resemblance was strengthened by the increased fragility of the erythrocytes. The usual progress of the disease is, however, very similar to that of splenic anæmia—splenomegaly in the early stages with fever, weakness, anæmia and latent icterus being followed by enlargement of the liver, and finally by cirrhosis with ascites. The changes found in the sections of liver and spleen examined, would correspond to that usually described as typical of Banti's disease. In this connection it is worth mentioning, that Eppinger and Walzel (1926) record that in an examination of a number of spleens removed, with good result, from patients suffering from

blood sugar falls continuously and the animals die with symptoms of hypoglycæmia, although the muscles contain sufficient glycogen to keep the blood sugar up to the normal level if it were released. These workers conclude that the circulating glucose is regulated by the liver and not by the muscles. The work of Cori and Cori (1926) on the disposal of glucose in normal rats points in the same direction. These observers have found that more ingested sugar is oxidised when insulin is given than when it is withheld, and that the source of this sugar is the liver. It would seem, therefore, that in normal animals absorbed glucose is stored in the liver and in the muscles, but that the hepatic stores are much more readily mobilised than those of the muscles. With regard to monosaccharides other than glucose it is generally accepted that lævulose is dealt with entirely, or almost entirely by the liver, although Mann (1925) has stated that this sugar can apparently be utilised in the complete absence of this organ. In normal animals, at all events, ingestion of lævulose causes very little rise in blood sugar and it is on this ground that the modern lævulose test for liver efficiency is based [MacLean and de Wesselow (1921)]. Another sugar which has been used for this purpose is galactose. Davies (1927) states that 40 grammes of this monosaccharide does not cause the blood sugar of a normal man to rise more than about 0.3 per cent. Folin and Berglund (1922) have also found that galactose is much less effective than glucose in producing hyperglycæmia in man, but Bodansky (1923) is of opinion that galactose produces a greater rise in blood sugar in dogs than glucose. It is possible that the ability of the liver to deal with this sugar varies in different animals.

A study was made of the carbohydrate tolerance in two well marked cases of muscular dystrophy (Erb's juvenile type). Both patients were Pathan males, aged respectively 17 and 18 years. They sought admission to hospital on account of muscular wasting and weakness which were symmetrical. Little in the way of family and personal histories could be obtained, but No. 1 (B) stated that he had suffered from marked weakness for about six months, during which time he gradually became unable to get up or to remain standing. The wasting began apparently in the thighs and pelvic girdle and extended to the arms. On admission it was most marked in the flexors of the hips, the extensors of the knees, the glutei, the spinal muscles and the muscles of the shoulders and upper arms. The muscles below the knees, those of the forearms and hands, the tongue, the masticatory and laryngeal muscles, the abdominal muscles and the muscles of the face were normal. Electrical excitability and loss of power were diminished in proportion to the wasting. There were no sensory changes and no fibrillary tremors. A moderate degree of *talipes equinus* was present. Patient No. 2 (S) was admitted in very much the same condition as No. 1, except that there was no *talipes*. This patient stated that he had noticed weakness and wasting of the muscles for about five years, the first disability being loss of power in standing and walking. In this case, however, it would appear that the disease began in the upper arms. Patient rose from the supine to the erect position in the manner typical of the disease. No abnormality other than

which is taken up by the reticulo-endothelial cells, has been found to result in the appearance of agglutinins against B proteus O X 19 in guinea-pigs infected with typhus fever (Singer, 1926). The experiments of Gay and Morrison (1923) led them to conclude that the natural resistance of rabbits to experimental streptococcus infection is largely, if not entirely, due to the local histiocytes. Gay (1926) later found that intrapleural immunisation led to an infiltration of the subserous layer of the pleura with mononuclear cells to such an extent that this layer was increased as much as forty times in thickness, and that bacteria introduced into the pleural cavity were rapidly taken up by these cells. Carrell and Ingesbrigsten (1912) showed that hæmolysins were produced *in vitro* when goat's erythrocytes were added to culture of guinea-pig's bone marrow or lymph glands, tissues rich in reticulo-endothelial cells. Malaria is one of the diseases in which there is enlargement and proliferation of the reticulo-endothelial cells, (especially of the spleen and liver) which take up red and white blood corpuscles, pigment and parasites (Gaskell and Millar, 1920). While the hæmoglobin set free by the parasites is undoubtedly an important factor in starting this reaction, it is not improbable that another stimulus is provided by the parasite itself. Proliferation of the reticulo-endothelial cells is found in such conditions as kala-azar, trypanosomiasis, typhus fever, histoplasmosis and Oroya fever (Sacks loc cit), diseases in which hæmolysis is not a prominent feature. It is known that the resistance of individuals exposed to chronic malarial infection varies considerably. If not properly treated some die from the direct effects of the disease, while others acquire partial or complete immunity. It is possible that a prolonged cellular immunity reaction is, in part at least, responsible for the condition we are dealing with, the hypertrophy eventually involving the fibroblasts. The response of these people to infection may be such, that immunity is only, if ever, attained by a degree of hyperplasia of the mesoblastic elements of the spleen and liver that interferes with the normal functions of these organs. Malarial infection might therefore persist beyond the stage of cirrhosis or be eradicated at this stage or earlier. Changes in corpuscular resistance are probably somehow related to the activity of the reticulo-endothelial cells, while cirrhosis is determined by production and multiplication of the fibroblasts. It would not therefore, necessarily be the case that increased resistance of the red blood cells in hypotonic saline would coincide with fibrosis of the liver although it often does. Eppinger (1922) believes that in cirrhosis of the liver, the agent damaging the parenchymatous cells also destroys the Kupfer cells, those which survive producing fibroblasts in great numbers, and that the reticulum cells of the spleen start a fibrosis of that organ. Sinton and Hughes (1924) showed that in ordinary acute cases of malaria there is some disturbance of hepatic function, and mention has been made of an acute hepatitis that attacks chronic malarial subjects in the Punjab in bad fever seasons. Injury thus caused to the liver cells might also be a factor in starting the cirrhosis. Glucose or cane sugar tolerance tests were done on three of the patients and revealed a defect of the glycogenic function of the liver in each case.

Date	Time	Blood sugar per cent	
15-1-27	10-0 a m	186	50 grammes of levulose orally
	10-30 "	221	Lævulosuria
	11-0 "	216	
	11-30 "	216	
	12-0 noon	191	
17-1-27	10-20 a m	156	1 c c of pituitrin subcutaneously
	10-50 "	191	Glycosuria
	11-20 "	202	
	11-50 "	166	
	12-20 p m	166	
18-1-27	9-0	186	50 grammes of glucose orally and 1 c c of pituitrin subcutaneously
	10-5 "	162	Glycosuria
	10-35 "	202	
	11-5 "	221	
	11-35 "	206	
25-1-27	9-15 a m	128	1 c c of adrenalin subcutaneously
	9-45 "	152	Glycosuria
	10-15 "	191	
	10-45 "	187	
	11-15 "	166	
26-1-27	9-20 a m	128	15 units of insulin subcutaneously
	9-50 "	109	
	10-20 "	100	
	10-50 "	090	
	11-20 "	117	
29-1-27	Output of creatinine in 24 hours—539.5 mg Weight 40 kilos Creatinine Co-efficient (mg per kilo per 24 hours)—13.5 (adult normal 20 to 25) Creatine as creatinine in 24 hours 376.7 mg Bilirubin content of blood serum—normal No urobilin in urine		

In this case also the fasting blood sugar level was generally high but varied considerably. The tolerance to glucose was defective and the renal threshold seemed to be about 190. Pituitrin again caused a hyperglycæmia and when given with glucose the blood sugar was higher at the end of two hours than when glucose was given alone. Lævulose (50 grammes) raised the blood sugar 30 mg in half an hour and the initial level was not reached in 2 hours. At a later stage the tolerance of this patient to 40 grammes of galactose was tested after treatment had been stopped for four days. The maximum rise was 35 mg which occurred in one hour, and the blood sugar returned to the original figure before  $1\frac{1}{2}$  hours. One c c adrenalin gave rise to a well marked hyperglycæmia which persisted for over 2 hours. Fifteen units of insulin caused a fall of 38 mg in  $1\frac{1}{2}$  hours after which the blood sugar began to rise. On the 2nd January, 1927, this patient was put on 2-grain doses of sodium nitrite three times daily with the object of increasing the blood supply to the muscles, as has been done by Cajori, Pemberton and Crouter (1926) in cases of rheumatoid arthritis. This was combined with 60-grain doses of sodium phosphate thrice daily, so as to ensure a plentiful

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 SINGER, E and H ADLER (1924) *Zeits f Immunitätsforsch*, XLI, p 468

produced by adrenalin (Burn, 1915), but in both patients pituitrin was followed by a rise in blood sugar. The effect of vaso-dilatation on the power to store ingested glucose and on the hypoglycæmic effect of insulin point to the defect being in the muscles. The degree of muscular wasting and hypotonia is illustrated by the low creatinine co-efficient in Case 2. The high renal threshold for glucose is difficult to understand. In neither case did the urine reveal any abnormalities on the part of the kidneys, and in Case 2, at all events, there was no retention of nitrogen. One may assume, perhaps, that the kidneys adapted themselves to the high level of sugar in the blood. The variation in the level of the fasting blood sugar in Case 2 probably indicates instability of the tone of the muscular arterioles and capillaries.

The lævulose tolerance curves in Case 2, both before and after the administration of nitrites and phosphates might be looked upon as evidence of hepatic insufficiency. There is considerable difference of opinion as to the extent of the rise in blood sugar after ingestion of 40 to 50 grammes of this carbohydrate which should be taken to signify abnormality. Spence and Brett (1921) looked upon a rise of 20 mg per 100 ccs of blood as indicative of damage to the liver, while Lollerman (1923) concluded that hepatic insufficiency is to be presumed when the height of the curve after 45 grammes of lævulose exceeds 135 per cent and the actual rise from the initial level is 30 mgs. King (1927) found that in ten normal people the average rise in the curve above fasting level after ingestion of 45 grammes of lævulose was 008 per cent. All observers emphasise the fact that in the normal the fasting level is reached in  $1\frac{1}{2}$  or 2 hours. Judged by these standards the lævulose tolerance curves of patient No 2 are distinctly abnormal. Other tests of the functional capacity of the liver, however, did not confirm this. Thus the bilirubin content of the blood serum was within normal limits, and so was the tolerance to 40 grammes of galactose. That the muscles do not take any considerable part in the storage of lævulose is shown by the absence of any effect of nitrites on the storage of this sugar.

### CONCLUSIONS

1 In two cases of well marked muscular dystrophy (Erb's juvenile type), the following defects in carbohydrate tolerance were found (a) high fasting blood sugar, and (b) defective power to store glucose.

2 Nitrites and phosphates were administered to one patient and resulted in an improvement in the glucose tolerance and in the general condition.

3 The cause of the diminished glucose tolerance is discussed and it is concluded that it is due to the wasting and poor blood supply of the voluntary muscles.

4 The renal threshold to glucose was high in both cases. In one patient the power to store lævulose was less than normal and was not improved by nitrites and phosphates. The tolerance to galactose was normal in this case.

We desire to express our thanks to Major V. R. Mirajkar, F.R.C.S., Professor of Physiology, King Edward Medical College, for permission to

# CARBOHYDRATE TOLERANCE IN TWO CASES OF MUSCULAR DYSTROPHY

BY

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AND

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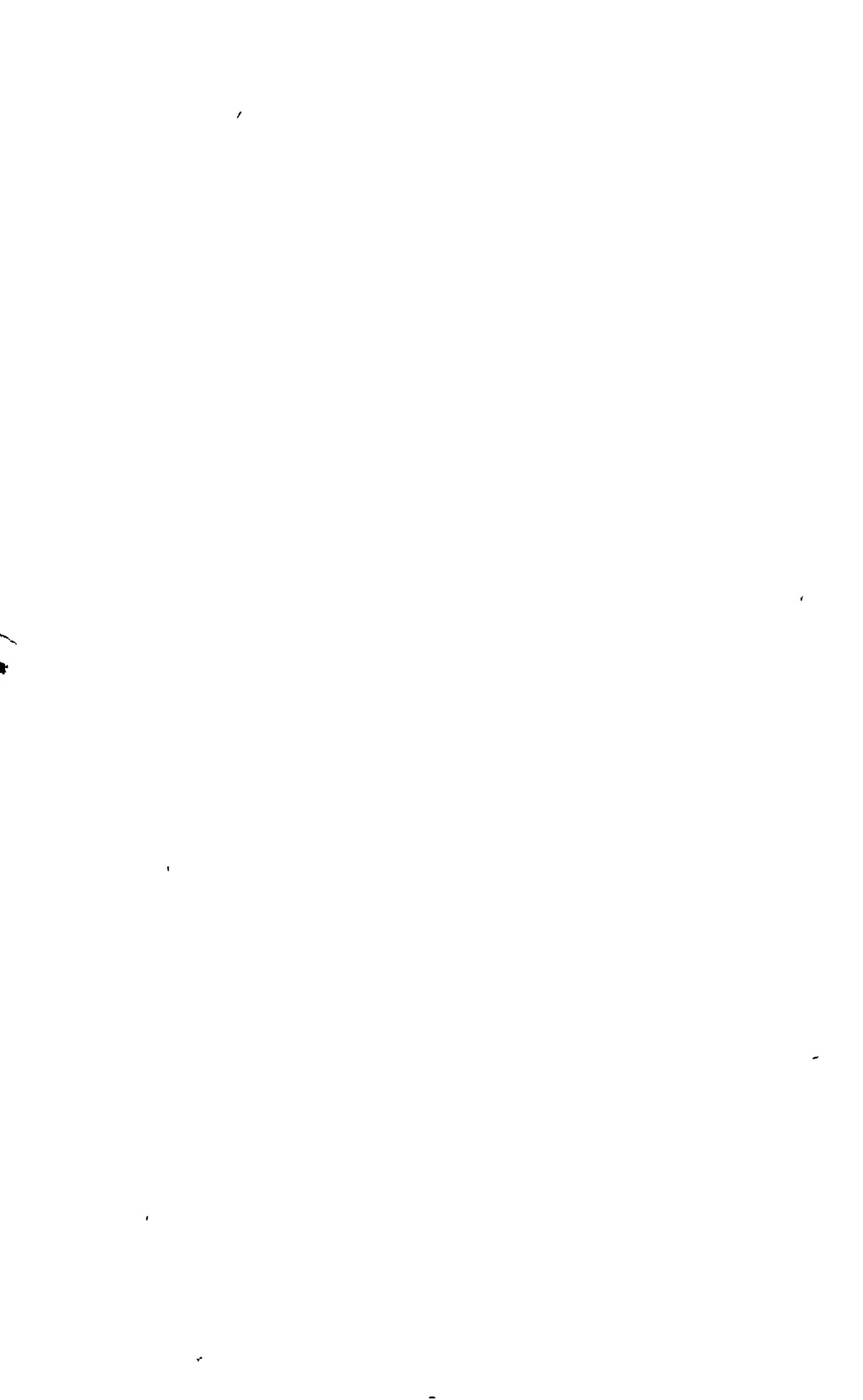
*(From the Department of Clinical Medicine, King Edward Medical College, and  
the Wards of the Mayo Hospital, Lahore )*

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RECENT work on the metabolism of carbohydrates has shown the importance of the rôle played by the voluntary muscles in the disposal of glucose. The investigations of A. V. Hill, Myerhof and their associates (Hill, 1926) indicate that the immediate source of muscular energy is carbohydrate. Cori and Cori (1925) have demonstrated on rabbits that insulin causes an increased rate of transference of glucose from the blood to the muscles, while the experiments of Lawrence (1926) on man have proved that muscular exercise greatly increases the hypoglycæmic effect of insulin, and burns carbohydrate and depletes the stores of glycogen in a way that insulin alone cannot do. Best, Dale, Hoet and Marks (1926) have accounted for the sugar that disappears from the blood of an eviscerated spinal cat under the influence of insulin when glucose is continuously infused by way of the veins. Some is burnt and some is deposited in the muscles as glycogen. Pemberton, Cajori and Crouter (1926) maintain that the low glucose tolerance of certain patients suffering from non-specific arthritis is due to defective blood supply to the muscles and other tissues, and they have demonstrated (1925) that the glucose tolerance in normal man is influenced by the amount of muscular tissue receiving an adequate supply of blood. The relative parts played by the liver and voluntary muscles in the regulation of the blood sugar level is indicated by the experiments of Mann and his co-workers (1925). In dehepatized dogs the





muscular was detected in either patient As far as they knew they did not belong to the same family The subjects were fasting at the beginning of each test and remained in bed on each occasion till the observations were concluded Blood sugar was estimated by MacLean's method

The following are the results of experiments on Case 1 (B) (only a few tests could be carried out as the patient left hospital)

Date.	Time	Blood sugar per cent.	
1-12-26	9-30 a.m.	171	50 grammes of glucose orally
.	10-0 "	211	No glycosuria
	10-30 "	236	
	11-0 "	227	
	10-30 "	207	
30-11-26	9-25 a.m.	181	50 grammes of lævulose orally
,	9-55 "	187	Lævulosuria
	10-25 "	190	
	10-55 "	177	
	11-25 "	172	
4-12-26	10-0 a.m.	171	1 c.c. pituitrin subcutaneously
	10-30 "		
	11-0 "	206	
	11-30 "	166	
	12-0 noon	156	
14-12-26	9-50 a.m.	177	50 grammes of glucose orally and 1 c.c. pituitrin subcutaneously
.	10-20 "	211	
	10-50 "	206	
	11-20 "	196	
	11-50 "	196	

The initial blood sugar was high on every occasion After 50 grammes of glucose it is seen to have risen to 226 mg per 100 c.c., and to have been well above the fasting level at the end of 2 hours The high renal threshold will be noted,—above 236 Fifty grammes of lævulose caused a rise of only 9 mg and the fasting level was reached before 1½ hours A c.c. of pituitrin (P D & Co), alone gave rise to a transient hyperglycæmia, while after glucose and pituitrin there was a smaller rise than after glucose by itself, but the level of the blood sugar still tended to remain above the initial figure

The results of some experiments on Case 2 are as follows —

Date	Time	Blood sugar per cent.	
14-1-27	9-45 a.m.	157	50 grammes of glucose orally
	10-15 "	211	Glycosuria
	10-45 "	257	
	11-15 "	211	
	11-45 "	166	

certain ascertained facts Thus retention of chloride occurs in azotæmic nephritis without retention of water, and Volhard and Fohr (1924) state that in the early stages of renal dropsy, there is actually concentration of the blood instead of dilution This fact indicates that the cause of the dropsy is extra-renal, the inability of the kidney to excrete water being only apparent, and attributable to a diminished amount of water in the blood-stream Some observers, notably Epstein, have associated the œdema with the diminution of the protein content of the plasma which is found in this condition, the diminution affecting the albumin more than the globulin According to Eppinger and Steiner (1917) this is due to the passage of protein from the plasma to the tissue spaces, while Mayrs (1926) thinks it probable that the loss is through the damaged renal glomeruli The views of Epstein on this point will be mentioned later However brought about, the result of such a change is a lowering of the osmotic pressure of the plasma, which is no longer able to prevent fluid being forced through the capillary walls by the blood pressure Loeb (1923), however, in a comprehensive discussion of the factors concerned in the production of renal œdema, comes to the conclusion that the distribution of water in the body is controlled rather by sodium chloride than by the plasma protein

Recently, de Wesselow (1925) investigated the capacity of the kidney for concentrating chloride after oral administration of KCl Four grammes of this salt dissolved in 200 ccs of water are administered without any previous food or drink restrictions The urine is collected hourly for 3 hours and the percentage of chloride in the samples is determined It is found that the maximum concentration, which is usually attained during the third hour of the test, is, in the vast majority of normals, 0.80 per cent or over de Wesselow has applied this test to the study of diseased conditions of the kidney and has come to the conclusion that nephritic patients showing primary œdema, fall into two groups In one of these, the œdema is extreme in degree and is associated with large effusions into the serous sacs especially the peritoneum There is abnormal permeability of the capillaries with passage of chloride and water into the tissue spaces, the chloride concentration of the tissue and ascitic fluid being higher than that of the plasma The output of chloride in the urine is low, not because of inefficiency of the kidneys, but because the chloride of the plasma has fallen to a low level, sometimes below the renal threshold The kidneys, therefore, do not get a chance of excreting normal amounts of chloride The œdema is of extra-renal origin and is associated with hypochloræmia and oligæmia, a combination which is also found in pneumonia, war-gas poisoning and extensive burns When the chloride content of the blood rises, the output of chlorides in the urine approximates a normal figure In the second group, which includes glomerulonephritis, eclampsia, pre-eclampsia, persistent œdema of the legs after acute nephritis, chronic nephritis complicating pregnancy and acute exacerbations of chronic nephritis, the cause of the œdema seems to be retention of water with secondary retention of chloride In some of these cases the plasma chloride is found to be at a high level after chloride ingestion In contrast

supply of phosphoric acid for lactacidogen formation. The patient stated that he felt better as a result of this treatment and after two months there seemed to be an increase of muscular power. When this régime had been in existence some time a further series of observations were made, the results of which are as follows —

Date	Time	Blood sugar per cent	
15-2-27	9-15 a m	182	50 grammes of glucose orally
	9-45 "	196	"
	10-15 "	171	"
	10-45 "	166	"
	11-15 "	128	"
9-3-27	9-15 a m	128	50 grammes of lævulose orally
	9-45 "	157	"
	10-15 "	152	"
	10-45 "	141	"
	11-15 "	141	"
7-3-27	10-0 a m	181	15 units of insulin subcutaneously
	10-30 "	122	"
	11-0 "	109	"
	11-30 "	109	"
	12-0 noon	090	"

The fasting blood sugar still remained at a high level and still varied from day to day. A marked improvement in tolerance to glucose was seen both as regards the degree and the duration of hyperglycæmia. The effect of 15 units of insulin was more marked than before, a fall of 91 mg of glucose per 100 c c of blood being produced and the level seemed to be still falling at the end of 2 hours. The ability to store lævulose was not improved as 50 grammes still caused the blood sugar to rise 29 mg in half an hour, and a rise persisted for more than 2 hours.

### DISCUSSION

The defective power to store glucose in these cases is probably the result of the wastage and poor blood supply of the voluntary muscles. Hector (1926) has demonstrated a diminished glucose tolerance in patients suffering from diphtheria and has attributed the defect to interference with the function of the liver, adrenals and nervous system. In these cases the initial blood sugar level was low. A lowered glucose tolerance has also been described in certain cases of post-encephalitis lethargica with normal or nearly normal fasting blood sugar [McCowan, Harris and Mann (1926a)]. Here the cause is probably excessive glycogenolysis from abnormal sympathetic stimuli, as is indicated by the fact that injections of hyoscine (1/100—1/50 grain) improved both the nervous condition and the ability to deal with glucose [McCowan, Harris and Mann (1926b)]. In the cases here described there was no evidence of nervous disease and the effect of pituitrin is against hyperfunction of the adrenals. This substance antagonises the hyperglycæmia

are also present. Thus Epstein states that the term nephrosis is applied to amyloid disease of the kidneys and to cases of glomerulo-nephritis with nephrotic manifestations. Dyke (1924) published an account of six cases of renal dropsy, in three of which there were no cardio-vascular changes and no retention of nitrogen. Microscopic examination of the kidneys revealed only slight changes in the glomeruli and interstitial tissue but extensive degeneration of the tubule cells. In the other three there were in addition to œdema, massive albuminuria, etc., hæmaturia, raised blood pressure and eventually uræmia. The kidneys showed not only degeneration of the cells of the tubules, but marked inflammatory changes in the glomeruli and connective tissue. Dyke concludes that the œdema is connected with the tubular degeneration, and the cardio-vascular manifestations and uræmia with the glomerular changes. Elwyn (1926), while admitting the occurrence of pure lipid nephrosis, thinks that the condition often follows inflammatory changes in the glomeruli.

The degenerative changes seen in the tubules in cases of renal dropsy seems to be of a lipid nature, the lipoids consisting chiefly of cholesterol esters. Some observers have found doubly refractive lipoids in the urine. It would seem that the 'myelin kidney' described by McNee (1922) and others is an extreme degree of the change found in this condition.

This communication deals with the results of an investigation into the nature of a number of chronic cases of renal dropsy admitted to the Mayo Hospital, Lahore, during the winter of 1926-27. Judging from hospital figures this condition seems to be fairly common in the Punjab. The patients all belonged to the lower rural classes and definite histories were difficult to obtain. They all seemed to have suffered from malaria for years and a history of dysentery was common. Many had enlarged spleens and in two there was also enlargement of the liver. None were suffering from ankylostomiasis. During the investigation three cases died in hospital, but post-mortem examinations were not allowed. The enquiry consisted of determination of the urinary changes, of the ability of the kidneys to excrete ingested chloride, urea and water, and of the concentrations of urea, chloride (as NaCl), and cholesterol in the blood. Blood analyses were carried out on samples obtained during the chloride concentration tests.

*Methods*—Blood cholesterol was estimated by the method of Leiboff (1924). This method is said to give results rather on the low side. The figures obtained on 10 normal persons ranged from 114.3 to 160 mgrms per 100 ccs of blood. The chloride concentration test was carried out according to de Wesselow (*loc cit*), chloride in blood and urine being determined by Whitehorn's (1921) method. The water test employed was that of Strauss and Grunwald which is founded on the fact that when a pint of water is given to a normal starving person and the urine collected at hourly intervals, the sum of the first three hours specimens is equal to the amount of fluid taken. When the kidneys are diseased the output is considerably less. Estimation of blood urea and determination of the capacity of the kidneys to concentrate urea were carried out by MacLean's (1924) methods.

work in his laboratory and to Dr Mohammad Yusaf for assistance during the investigation

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Patient No 16 died from uræmia 3 days after the reported observations were made. Blood urea was high and both concentration tests showed impaired renal function. No 9 also died in hospital about 3 weeks after admission. Blood urea was relatively low 3 weeks before death but rose to a high figure just before the final uræmic convulsions. Blood chloride was higher than in No 16, a fact which may account for the higher output of chloride in the urine. In both these patients the urine contained red blood cells. In No 14 the œdema began during the last month of pregnancy five months before admission, subsided to some extent after parturition, but never completely cleared up. Later some ascites developed. The chloride content of the ascitic fluid is seen to have been higher than that of the blood, but the latter is still well above the threshold level.

In patients Nos 5 and 15 there was no ascites and the degree of œdema was not very marked. The urine was loaded with albumin, small in amount and contained no red blood corpuscles. The concentration capacity of the kidneys for both urea and chloride was normal and the blood urea hardly raised. The blood cholestrol was low.

### DISCUSSION

Of the patients in whom a raised blood cholestrol content was found, only one (No 4) can, we think, be regarded as a case of pure nephrosis. This patient exhibited all the characteristic features of this condition as far as could be ascertained. The basal metabolic rate could not be determined, but the tolerance of the patient to large doses of thyroid combined with a high protein, low fat diet and the improvement that resulted from this line of treatment, indicated an absolute or relative deficiency of the thyroid gland. No 2 differed from No 4 in the comparatively poor concentration of chloride which was followed later by a falling off in urea concentration. It looks as if in this case nephritic or inflammatory changes were gradually complicating the nephrotic process.

In Nos 6 and 11 inflammatory changes were far advanced but the blood cholestrol remained high. It cannot be said whether in these patients the disease began as a pure nephrosis and later took on nephritic characters, the result of secondary contracted kidneys, but it is evident that there was a nephrotic element present. Blood for cholestrol and chloride estimation could not be obtained from No 3 owing to the degree of œdema, but the other features of this case suggested a pure nephrosis. In the group of cases made up of Nos 1, 8 and 13 the blood cholestrol, while not above normal, was fairly high. It is doubtful, however, if these can be looked upon as complicated cases of nephrosis, although this condition may have been present at an earlier stage. The group consisting of Nos 7, 9, 10, 12 and 16 exhibited varying degrees of renal insufficiency and contained two of the three fatal cases of the series. It is noteworthy that these and No 11 are the only patients in whose urines red blood corpuscles were found.

In cases Nos 5 and 15 the cause of the dropsy is not to be found in a disordered metabolism, in a passage of water and salt from the blood to

# A STUDY OF RENAL ŒDEMA

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CHRONIC nephritis is usually seen in one of two forms (1) a type characterised by high blood-pressure, cardiac hypertrophy, retention of nitrogen and uræmia, but without dropsy until heart failure occurs, and (2) a form in which œdema is a marked feature from the start. In the former there is inability to secrete a concentrated urine with consequent polyuria, or at times suppression of urine. Urea concentration is low and albuminuria slight. In the pure œdematous variety there is no inability to concentrate urea, no nitrogen retention and no tendency to uræmia. The urine is small in quantity, of high specific gravity and contains a large amount of protein. There is, therefore, little evidence of renal insufficiency apart from œdema and albuminuria. Microscopically, the kidneys show little or no evidence of inflammation, the changes found being degenerative in character, especially affecting the renal tubules. Widal (1912) attributed the œdema in these cases to chloride retention with secondary retention of water so as to maintain the chloride content of the body fluids at a level compatible with life. Hence this type of kidney disease was called hydramic or hydropiginous in contrast to the non-œdematous type which was termed azotæmic. While pure examples of one or other of these varieties are common, mixed cases are frequently seen. The disease may begin in one form and gradually change in type or it may retain most of its own characters and take on in addition those of the other variety. Widal's explanation of renal œdema was at first generally accepted, but it was gradually found that it did not fit in with



TABLE--(contd)

URINE FINDINGS							BLOOD FINDINGS			REMARKS
Urea concentration (percentages)			Hourly output of chlorides in mgrms NaCl per 100 c.c of urine after ingestion of 4 grms KCl				Urea in mgrms per 100 c.c. of blood	Cholestrol in mgrms per 100 c.c of blood	Chlórides in mgrms NaCl per 100 c.c of blood	
1	2	3	1	2	3	4				
08	14		360	480	220	260	102	100	460	
10	14	16	320	410	340	260	44	100	640	
12	13	15	100	160	120	180	38	111 F	480	
19	21	3.3	1,400	1,620	1,760	1,860	50	100	480	

to these two groups of oedematous cases, patients with pure azotæmic nephritis show retention of chloride in the blood without any retention of water, the inability to excrete chloride being primary and parallel to the inability to excrete urea. de Wesselow concludes that 'it is doubtful whether true dissociation of the concentrating capacity of the kidney for urea and chloride exists'.

Of late there has been a tendency to look upon renal disease in which only purely degenerative lesions of the kidneys exist as something apart from the ordinary nephritides where inflammatory changes are the chief feature, and the term 'nephrosis' or 'lipoid nephrosis' is being used to indicate the clinical condition characterised by massive albuminuria, oliguria, gross oedema and effusions into the serous sacs, reduction of the plasma proteins with relative increase of globulin, high blood cholesterol, normal blood urea and non-protein nitrogen and no cardio-vascular changes. In a paper by Izod Bennett and others (1927), which appeared during the course of the work here recorded, there is given a short history of the development of this conception. It has been brought to the notice of the English-speaking world largely through the work of Epstein. In a recent paper (Epstein, 1926) this worker draws attention to the high tolerance to thyroid extract and thyroxin exhibited by subjects of this disease. The basal metabolic rate is lowered in about 60 per cent of cases but not more than 10 to 20 per cent as a rule. In myxoedema, the B M R tends to fall to a uniform level of minus 40 (Kendall, 1919) yet nephrotic patients tolerate, and are benefited by doses of thyroid many times greater than the amounts tolerated by myxoedematous cases. Doses of 15 to 60 grains are sometimes required to produce a metabolic effect. Epstein points out that a relationship seems to exist between the B M R and the cholesterol content of the blood. Conditions which lower the B M R cause an increase in the blood cholesterol, while those which raise the rate of metabolism cause a decrease. This relationship is found to exist in myxoedema. He considers that 'the extra-renal factors which are so striking and unique in chronic nephrosis are the expression of a primary systemic disorder which affects first the protein and later the lipoid metabolism'. Although the activity of the thyroid gland may be normal or even increased, it is not able to meet the demands created by the metabolic disturbance. 'The tubular degeneration in the kidneys is the result and not the cause of the metabolic change and Epstein suggests the name 'albuminuric diabetes' for the condition. In treatment, he uses a high protein (2—3 grms per kilo), low fat diet to replace the protein loss of the blood plasma, to compel the tissues to utilise protein and to reduce the lipoidæmia. When dietary measures are not sufficient thyroid extract or thyroxin is used to stimulate protein metabolism. He maintains that certain cases of chronic nephrosis are susceptible of complete cure by these methods, but that this may take a year or more to accomplish.

While it is admitted that in certain cases of renal dropsy with hypercholesterolæmia only degenerative lesions of the kidneys are found, many observations show that in some such cases, inflammatory and other changes

2 In two cases primary retention of water seems to have been the cause of the œdema

3 In the remaining cases it is probable that the dropsy was of pre-renal origin, due to abnormal capillary permeability to water and salt

4 Of the latter, one patient exhibited the features of pure lipoid nephrosis, and three those of nephrosis complicated by inflammatory renal changes. Incomplete investigations on another case pointed to a condition of pure nephrosis. In the remainder the findings indicated degenerative and inflammatory changes but the cholesterol content of the blood was not raised

5 In a case of pure nephrosis and in one of nephrosis with very early nephritic changes considerable improvement was effected by a high protein diet and large doses of thyroid extract

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*Results*—Clinical notes and a summary of the results obtained on each patient are given in the table. Cholesterol, chloride and blood urea are expressed in mgrms per 100 ccs. The figures under urea concentration are percentages of urea in the urine excreted during the 1st, 2nd and 3rd hours respectively, after ingestion of 15 grms of urea.

In four patients, Nos 2, 4, 6 and 11, the blood cholesterol content was above normal while the figures are at the upper limit of normality in Nos 1, 8 and 13. Nos 2 and 4 remained in hospital for some time and were given a diet containing much protein, little carbohydrate and no fat, combined with thyroid extract (B. W. & Co.) in daily doses of 8 grains rising rapidly to 18. Neither showed any symptoms of hyperthyroidism. The blood cholesterol fell in each case and there was marked clinical improvement. In case No 2 the blood urea is seen to be above normal, and in this patient the power to excrete urea diminished with time. The response to the chloride concentration test was only fair, although the blood chloride was above the average normal (480 mgrms NaCl per 100 ccs), and therefore above the ordinary threshold value. de Wesselow mentions that the usually accepted threshold level of plasma Cl is 340 mgrms per 100 ccs. This would correspond to a total blood NaCl content of about 450. In this patient therefore, it is probable that the cause of the defective chloride excretion lay in the kidneys. In case No 4 both urea and chloride concentration continued to be normal as the blood cholesterol fell, although there is some rise in the blood urea. This may be due, as is pointed out by Epstein, to the rise in blood concentration which results from the re-establishment of normal metabolic processes. In No 6 the blood urea was high and the concentration of both urea and chloride was low. The blood chloride was also at a low level. Patient No 11 died from uræmia three days after admission. Examination three days before death revealed very defective concentrating capacity of the kidneys for urea and chloride. Blood urea was not estimated, but the blood chloride was found to be above the average threshold level. The urine contained a large amount of albumin and some red blood corpuscles and the blood pressure was raised.

In patient No 1 the concentrating power of the kidneys for urea is normal in spite of a fairly high blood urea content, but the response to the chloride test is poor. The cause of this is indicated by the high level of NaCl in the ascitic fluid and low level in the blood—below the renal threshold. In No 8 the concentrating capacity for urea was fairly good but the output of chloride was poor, although the blood NaCl was above the threshold level.

In Nos 7 and 12 the response to the urea and chloride concentration tests was fair. The latter test gave higher figures in No 12 than in No 7, possibly because of the higher figure for blood chloride. The blood urea was high in both, especially No 7. The urines of both patients contained albumin in large quantities. Red blood corpuscles were also present. In case No 10 the oedema seems to have been first noticed during pregnancy, but when the patient came under observation the clinical condition and pathological findings resembled those of Nos 7 and 12 except that the blood urea was low.

72 per square mile, and is less fertile than the northern area, cultivation being for the most part confined to the valleys of streams

In the plateau, 85 per cent of the population are Hindus—Baghels, Rajputs and lower castes, while in the hills the population are chiefly animist hillmen, the Gonds

The average rainfall in the 'Uparihar' is 42.2 inches a maximum of 64 inches was recorded in 1894, and a minimum of 26.34 inches in 1905, which was a year of shortage of crops and of famine

With the exception of coal mines at Umaria and Burhan in the hill country, and of the lime kilns at Sutna, the sole occupation of the State is agriculture

In the years of normal rainfall it is self-supporting as to cereals, and exports grain in considerable quantities, and only in a year following a failure of the monsoon rains is the normal outflow of grain exports replaced by an inflow of imported cereals. Sutna, on the G. I. P. Railway, is the distributing centre and commercial emporium of the State. A metalled road, 31 miles long, connects it with Rewa, the capital, from which radiate other metalled roads whose mileage aggregates 100 miles only. Apart from these, unmetalled roads and village cart tracks are conspicuously lacking, and owing to this lack of communications the villagers of the interior live in the circumstances and under the economic conditions which have prevailed since time immemorial. They are dependant for their food supply on their own resources, and the shortage of food-grains which follows a failure of the monsoon rains in a country depending for rainfall on its crops, is not capable of relief by imported grain, and is a vital feature in the subject under consideration

The land owners are Brahmmins, Baghels, Rajputs and others. The field work is done by labourers, the *harwalas*, who belong to the lower castes, Kols, Chamars, etc. With the field labour is associated a form of economic bondage, the '*harwaha*' or '*lugwa*' system, which is relevant to our subject

A '*lugwa*' is a low caste labourer who having incurred a debt of some Rs. 60 to Rs. 80 by extravagant expenditure on a marriage ceremony, or such like, has pledged the services of himself and his family to a landowner until such time as he has paid off his debt. His master, in return for these services guarantees his bondman's food and clothing. The food is a '*bharwar*'—a payment in kind of a daily ration of such foodstuffs as are available from the '*kharif*' and '*rabi*' crops and as may be expected under these circumstances, the '*lugwa*'s' ration consists of the cheapest grains. It is unvaried and monotonous and they are the first to feel the stress of scarcity

Grazing grounds are abundant in area, although poor in quality, and the cattle are small and degenerate. In a Hindu State none are killed for food, and no effort is made to control their reproduction or to eliminate the unfit. The average village cow yields only about  $\frac{1}{4}$  seer of milk per day. Only the better classes drink milk or consume dairy products, and little enough at that, and such of the poorer classes as have cattle collect their milk for the preparation of ghee, which, being easily portable, is sold to obtain the money which pays the land revenue of the State. The only milk product which is consumed by them is '*matha*'—butter-milk. There is a noteworthy scarcity of fruit and of the leafy vegetables which

Patient.	Patient.	Date	Clinical notes	URIN		
				Amount in 24 hours in oz	Water test in oz	Physical examination
2 J, æt. 25 years	7 S D, æt. 35 years	2-12-26	Blood pressure 100-75 History of malaria and dysentery Swelling of face, limbs and abdomen for five months On admission generalised œdema and ascites	36	10	Very marked albuminuria Specific gravity 1010 RBC and pus cells Granular and epithelial casts
1 J D, æt. 50 years	9 N S, æt. 10 years	21-1-27	Blood pressure 155-100 Gradual onset of œdema during six months On admission marked œdema and ascites necessitating frequent tapplings œdema and ascites increased in intensity Died of renal failure on 12-2-27 Blood urea being 109 shortly before death	..	3½	Very marked albuminuria Specific gravity 1016 Granular and hyaline casts Red blood cells
8 K A, æt. 30 years	10 G P, æt. 30 years	9-2-27	Blood pressure 120-90 œdema of face and legs for five months which began during pregnancy and continued after chiefly No ascites Spleen 2" below the costal margin		10	Marked albuminuria RCB and leucocytes present
13 S S, æt. 15 years	15 R, æt. 25 years	11-2-27	Blood pressure 100-70 History of malaria and dysentery œdema for one month On admission œdema of face and lower extremities No ascites	24	4	Albumin in small amount Granular casts and leucocytes present

The pulses ripen in August and September, and the cereals, Kakun and Saman, ripen first about September or October, followed by Kodon, Bajra, rice, and last of all, Jowar in November. The method of preparation of these cereals for food is relevant to this enquiry.

Kodon is husked with the 'chakara' a soft grind stone made of sun-dried clay held together by rice husks. It is then cleaned in the 'supa' or winnowing basket and pounded with the Musal (the pestle) in the 'kari' or mortar, and the husk—'kanna'—is given to animals or may be eaten by poorer classes. The resulting gram is known as 'Kodai'. This is washed and boiled, like rice, for eating as 'Kodo ka bhat' and the thick rice-water in which it is boiled, is fed to cattle.

Saman and Kakun are similarly treated, Jowar and Bajra, after being trodden by oxen, and winnowed, are ground in a hard stone Chakki and the resulting whole meal is cooked over a girdle ('Tawa') into a girdle cake or 'roti'. Rice is reaped in October, trodden in the 'Kalihan' and winnowed by the 'supa'. The gram is husked in the 'chakara,' shaken in the 'supa' to separate the outer husk, which is used to consolidate clay for bricks. The grain at this stage is known as 'Bagri'. It is now pounded in the 'musal' and 'kari' to remove part of the pericarp. It is now 'Chawal,' and the bran, 'kanna,' is given to animals. It is not polished or parboiled. It is eaten boiled, in the usual manner. It will be seen that a very varied selection of cereals is available from the 'kharif' crop for the use of the lower classes, and that its method of preparation is not such as seriously to lower its vitamin content.

At this time of year, the poorer classes use small quantities of green leaves of a wild plant, 'Chakaora,' which grows in the rains and is gathered and used in August and September while still soft. It is eaten with salt, condiments and vegetable oils.

No other green vegetable food is available until 'Chuna ki bhaji' comes into general use in December. This consists of the green shoots of the young Chuna plant, gathered when it is big enough to admit of the young shoots being 'pruned' by being nipped off by the finger and thumb. This prevents the plant getting stalky and induces a branching which improves its yield. These prunings are eaten raw with salt, or cooked, or dried for storage, and appear to be a valuable source of vitamin A, *see* later.

Common salt is used by all, as required. The vegetable oil, mustard oil, and linseed, are used occasionally in small quantities by the poorer classes, with these vegetable dishes, on the rare occasions when they are available. Their inability to supply fat soluble A is the reflection with which one regards them as a dietary ingredient.

The 'rabī' cereal crops consist of the following —

Wheat	<i>Triticum aestivum</i>
Barley	<i>Hordeum vulgare</i>
Matra	<i>Lathyrus sativus</i>
Chuna	<i>Cicer arietinum</i>
Masuri	<i>Ervum lens</i>

the tissues nor in inability of the kidneys to excrete chloride. Primary retention of water would seem to be the cause. These cases would therefore belong to de Wesselow's second or hydræmic group.

In Nos 1 and 14 the chloride content of the ascitic fluid was determined and was found to be higher than that of the blood. This indicates increased permeability of the capillaries to chloride and water, the characteristic of de Wesselow's first group. That the remaining cases also belong to this group is indicated by the degree of œdema, pronounced ascites and general features. In Nos 4, 14 and possibly 3 chloride concentration was normal because of the high blood chloride and good concentrating capacity of the kidney as revealed by the urea test. In No 1 the low output of chloride was probably determined by the low level of chloride in the blood. Low blood chloride combined with renal insufficiency caused the poor response to the chloride test in Nos 6, 7 and possibly 10 and 16, while in Nos 2, 8, 9, 11 and 12, in whom the level of the blood chloride was well above the kidney threshold, renal defect was alone responsible. It will be noticed that in case No 2 the power to excrete chloride was impaired earlier than the power to excrete urea, but that later both functions came into line. In No 13 also urea concentration was normal at a time when chloride concentration was poor. It may be that the capacity of the kidney to concentrate chloride is more easily upset than the capacity to concentrate urea.

In the cases that ended fatally it was noticed that there was a marked increase of the œdema shortly before the onset of uræmia. The cause of this is indicated by the results of the chloride concentration tests carried out on Nos 11 and 16 a few days before death. There was very pronounced inability on the part of the kidneys to excrete chloride, with consequent passage of increased quantities of salt and water through the permeable capillaries. The response to the water test was defective in all cases and did not, on the whole, furnish any information of value.

In view of the good results, temporary though they probably were, obtained in patients Nos 2 and 4 with thyroid administration and high protein diet, it is important that cases of uncomplicated nephrosis should be detected early. For this reason, we consider that in all chronic cases of renal dropsy, an estimation of the blood cholesterol should be carried out as well as a urea concentration test. The latter may be confirmed by de Wesselow's chloride concentration test, but this does not seem to be essential, and cannot be properly interpreted without an estimation of the chloride content of the blood and of the ascitic or œdema fluid.

### CONCLUSIONS

- 1 The results of an investigation into the nature of 16 chronic cases of marked renal œdema are reported. In addition to clinical observations, determination was made of the capacity of the kidneys to concentrate urea and chloride, of the amount of chloride (as NaCl), urea and cholesterol in the blood, of the ability of the kidney to excrete water and of the urinary changes.



a knowledge of the paucity of such fare in the famine year in which, perchance the case had developed

Those who can afford to be more fastidious in their feeding consider the 'matra roti' to be hard, difficult to masticate, and indigestible, while they know its proneness to produce lathyrism. Ordinarily the better class household uses it as a 'dhal,' taken with rice in the evening meal, and under such circumstances it never reaches one-third to one-half proportion of the diet, which Buchanan and other observers agree to be dangerous. There are areas, however, where its predominance in the crops entails its being eaten in large quantities mixed with wheat or barley as a 'roti' (*see* notes on Kotar, where the amount of matra eaten is large, and the number of cases is considerable). For eating, matra is ground, unhusked, in the 'Chakkı,' to form a yellow flour which is laked into a 'roti,' eaten without sieving by the lower classes. Better class people may remove the husk from the flour before preparing the 'roti.' As a 'dhal' it is like the other pulses, husked in the 'Chakra,' which breaks the shell and turns out the yellow hemispherical endosperm, which is separated from the husk by shaking in the 'supa,' and boiled and eaten with rice.

Thus matra, and now for its ill effects

### CLINICAL

The clinical manifestations of the disease have been recorded in great detail by earlier observers, who had, moreover, the opportunity in famine years, of seeing large numbers of cases, of recent origin. Continued search has, in the course of this investigation, revealed only 13 cases which occurred in 1926. Examination of old cases might have been multiplied *ad lib*, but it was found that information as to diet and history of onset could not be obtained from the old outstanding cases and it seemed useless to cover ground already traversed by earlier observers.

Brief notes, taken in the field, have been recorded as to 56 cases, old and new, from which the following observations may be deduced

#### *Age of Onset*

In the old standing cases, it is not possible to ascertain with any accuracy the age of the patient at the time of onset. Out of 23 cases that occurred in 1924, 1925 and 1926, the following was the incidence by decennial age groups —

5 to 10 years	4 cases
11 to 20 "	9 "
21 to 30 "	7 "
31 to 40 "	3 "

Buchanan (1904) who saw a large number of cases, places the onset in the majority of cases in the groups 10 to 20 and 21 to 30. The villagers remark that it is always the strongest and physically active who is struck down.

#### *Sex*

Few female cases were brought for exhibition, but the figures given for Kotar, Abair and Kuan (qv) based on a census, give a fairly reliable estimate of the relative sex incidence

# A FIELD STUDY OF LATHYRISM

BY

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*'Karya matra, pir pisan  
Uske khac, gor nisan  
Hale chandi, matke kul  
Ye dekho matra ke shul'*

Translation —

*'The black pea, with its yellow flour  
From eating it comes trouble in the legs,  
Flapping top knot and swaying hips,  
Behold the ill effects of eating matra'*

## INTRODUCTORY

THE writer was deputed under the Indian Research Fund Association in November, 1926, to examine in the field the epidemiological and clinical aspects of lathyrism

Bundelkhand and Baghelkand in Central India, being areas in which a large number of cases had been located, were selected as the venue of the investigation, with Rewa, and later, Sutna, as its headquarters. The investigation was pursued by daily visits to all likely villages within five to ten miles of a motoring road

## REWA, General

Rewa State, the scene of the greater part of these observations, is a treaty State in the Baghelkand Political Charge of the Central India Agency, of which charge it is the largest and most westerly State. It has an area of about 13,000 square miles, about the size of Bulgaria. A range of hills divides it into two natural areas. One, the northern area, consists for the most part of an upland alluvial plain, the 'Uparihar,' about 1,000 feet above sea-level, which is continuous with the main Central Indian plateau, it is open cultivated country, with a population of 176 per square mile

The southern area consists of hills which culminate in the peaks in which rise the Sone and the Nerbudda rivers. It is forest-clad, has a population of

relieved the monotony, such being beyond their means. Occasionally the 'nicheia matra' or unmixed lathyrus, might be varied with a 'berra,' a mixture of wheat and lathyrus, or 'jao berra,' barley and lathyrus, in which lathyrus would predominate. Another mixture, that of two legumes, gram and matra, seemed particularly noxious, and the 'jao berra' seemed more dangerous than the wheat berra. A fifty-fifty mixture seemed to be the dangerous proportion, which agrees with Buchanan's observations (Buchanan 1904). It was interesting to contrast this pre-lathyrism diet of the poorer class patients with that of the household of their employers, the Brahmins. Thus, in a Brahmin landowner's household of 6 persons, 4 adults and 2 children, with two lathyrism-lame 'lugwas,' all eating the same matra, about 5 seers of grain would be used daily. The basis of the morning meal would be a 'roti' of wheat and of the evening meal 'dhal' and rice the 'dhals' being of various pulses, among them lathyrus, which would be used in April, May and June, at other times 'arhar,' 'mung,' etc, would take its place. This household had 7 buffaloes and 12 kine. About  $1\frac{1}{2}$  seers of milk and 1 chhatak of ghee was used daily, the rest of the ghee being kept for sale. The buttermilk was used in the household and issued to the 'lugwas'. No fruit was consumed except mangoes in their season, and green vegetables would occasionally be purchased from a Kachhi. The wild vegetable 'chorai' would be gathered and used in its season. Pumpkins, rhuia, etc, 'chuna ki bhaji' in its season, salt, and other condiments, vegetable oils, and mahua flowers, fresh and dried. Contrasting the diet of a family of a caste and station of life which ordinarily escapes lathyrism except in a famine year, with that of the low caste lathyrism patient, one is struck by the absence from the diet of the lathyrism cases of the substances known to be rich in fat soluble A, viz, milk, butter and the fresh green leafy vegetables.

### *Mode of Onset*

It is known that an attack of fever may precede the appearance of the symptoms of lathyrism. 14 out of 51 cases gave a history of an attack of an intermittent fever, with rigors and sweating, lasting for 5 to 6 days to a month, in the autumn, after recovering from which they found themselves lame. The time of year, the type of fever, and the treatment, 'upas' (ten days' starvation, which is ordinarily the village treatment of malaria), are suggestive of malaria having been the cause of the fever. Three cases gave a history of an attack of dysentery having been an immediate antecedent of their lameness, one of an attack of smallpox, while in another, a medical man gave me a definite diagnosis of pneumonia having been an antecedent. In ten cases, in whom no fever had occurred, an exposure to cold or wetting, or unusual exertion had been an immediate antecedent. Thus one man had been on 'begar' (forced labour) on a 'sarkari' bund with short rations, for eight days, with rain falling. Another had been weeding a rice field in two feet of water or planting 'singhara nut' in a tank, roofing a house, chasing run-away cattle and so on. The onset is always dramatically sudden and unusually unheralded by prodromata. A typical history would be that the patient had had a heavy day's ploughing in heavy rain and had taken

are cultivated and used in other parts of India, and only the better class village family obtains at rare intervals and on feast days some green vegetables, which are grown and hawked by itinerant 'Kachhis' or vegetable-sellers. Mango groves abound, and the mango in its season as the green or ripe fruit, as a decoction called 'panna,' or a dried and powdered condiment—'amchui,' is largely used. Mahua flowers are also used to a small extent in the 'Uparihar,' but their vogue,—fresh, fermented, or sun-dried,—is greater among the Gonds of the hill tracts, who live on a variety of other jungle products which include the leafy vegetables. The Gond, unlike the orthodox Hindu of 'Uparihar,' does not grow any appreciable quantity of Lathyrus (*Rewa State Gazetteer*, 1907), and lathyrism is unknown among these hillmen.

### *Agriculture in Rewa*

As has been noted, the Rewan crops are entirely dependant on rainfall.

There are no irrigation canals. A primitive water lift, the 'Dhenkuri,' is used to irrigate from small kutchra wells the exiguous patches of green vegetables, tobacco, or sugar-cane, raised by the 'Kachhis,' but of the agricultural irrigation of the cereal crops from wells, as practised elsewhere, there is none. To conserve the rainfall and keep the fields moist enough to yield a 'rabi' crop, earthen banks styled 'bāndhs' are thrown across the lower ends of sloping fields to retain in them as much moisture as possible. The Rewan farmer recognises several kinds of soil, to which frequent references were met in one's wanderings. Thus there is the 'Mair,' a rich dark loamy soil, retentive of moisture and fertile in the autumn. 'Sigma' is a lighter soil more suitable for the 'kharif' crops and for rice. 'Dumat' soil contains the properties of both and may be 'Dufash'—suitable for both 'rabi' and 'kharif' crops, while there are other poorer soils, suitable only for 'kharif' crops and after years of rest. There are two harvests, in the spring and summer, the 'kharif,' watered by the monsoon rains, and the autumn-winter crops, the 'rabi,' dependant on the residual moisture in the soil. The cereal 'kharif' crops are as follows—

Rice	.	..	<i>Oryza satwa</i>
Saman	.	..	<i>Panicum frumentaceum</i>
Jowar	..	..	<i>Jorghum vulgare</i>
Kakun	..	..	<i>Sitania italica</i>
Bajra		..	<i>Pencillaria spicata</i>
Kodon	..	..	<i>Paspalum stoloniferum</i>
the pulses { Urad		.	<i>Phaseolus raduatus</i>
{ Mung		.	<i>Phaseolus mungo</i>
{ Arhar		..	<i>Cajanus indicus</i>

besides non-cereals, such as cotton and oil-seeds. Of the above list, the poorer classes use Jowar, Bajra, Kodon, Saman and Kakun, and the rice is for the most part appropriated for the use of the well-to-do.

These crops are sown during the rains, much ploughing is done while rain is falling, and the wettings and exertion the ploughman then experiences often determine the onset of lathyrism, as we shall see later.

The latter disorder furnishes the earliest sign of the disease, which can be detected in very slight cases showing an almost imperceptible impairment of locomotion

A normal gait is on a fairly wide 'wheel base,' to speak in motoring terms, whereas the lathyrism case, owing to adductor overaction, walks on a linear foot track, as if he were walking on a rail. He has no difficulty in heeling and toeing a line, unless rigidity and weakness interfere (Plate XLVI, fig 7)

The next thing that attracts attention is the progression with slightly bent knees, owing to overaction of the hamstrings

Overaction of the *gastrocnemius* and *soleus* leaves the toes on the ground when the heels have left it and leads to a dragging of the toes as the foot is advanced. In the effort to achieve this movement, the pelvis is tilted, while to maintain the balance disturbed by the linear foot track, the head sways from side to side. Though a slight case may walk unaided, one stick or two sticks may be required, according to the degree of disability. In more marked cases adductor spasm leads to a cross legged 'scissors gait' (Plate XLV, figs 5 and 6) and the calf muscles draw the feet into the position of spastic equinus, in which the patient walks like a ballet girl on tip toe (Plate XLIII, fig 2 and Plate XLIV, figs 3 and 4). In the last and extreme stage of the disease the postural reflex is entirely disorganised, the *quadriceps extensor* is overcome by the flexors, the legs give way at the knee joints and the patient is reduced to crawling in a sitting position with wooden hand supports (Plate XLIII, fig 1)

Except in these crawling cases of many years' duration in whom contractures from disuse prevented extension of the knee joints, it could be seen that this disorder of function is postural. A spastic equinus case can get down on the sole of his foot when standing at rest, and the spastic muscles can all be coaxed into relaxation by the pressure of the hand when the patient is in the recumbent position.

The degree of disability varies from the slightest possible malfunction indicated by a bent knee and a linear foot track, to one in which the patient is totally unable to walk. Notes on cases illustrating these different degrees of disability are sub-joined —

### *Crawlers*

Two such cases were seen. Stockman (1917) remarks that the clinical condition of such cases has not been recorded and the field notes may therefore be given in full

### *Notes on Cases*

*Case No 46*—Ram Das, male, age 30, caste Gosam, village Kotar. A professional beggar and lives on the alms of food grains which he collects. His mother is also a lathyrism case of old standing. He got lame 7 years ago (in 1920—a famine year) in the month of Sawan (July-August). After an attack of fever which lasted some days he felt some pain in the back and the legs. He awoke one morning to find himself totally unable to walk, and has remained so. No history of incontinence of urine or *fæces* could be elicited from him. Before he fell ill, he was eating a mixture of *matra* and barley got as alms in the village.

*Present condition*—He is totally unable to walk and crawls in the seated position with wooden hand supports. Knee jerks ++. Ankle clonus present in both feet. Plantar reflex active and markedly extensor. Tendon jerks obtained in *tendo achilles*, adductors and

These are sown in September and October and ripen in March and April. Double or treble sowing is practised, thus wheat is sown with gram and with 'matra,' in drills, the seed being fed through a tube ('nali') attached to the plough, through which the seed is dropped into the furrow. It is thus easy to distinguish what is sown intentionally and what is self-sown ('lamera'). Matra (*Lathyrus sativus*) is sown with the wheat, as a precaution against the wheat failing to germinate in a dry year, in which case only the matra comes up. It is also very largely self-sown. The resulting crop of such an yield of mixed sowing is known as 'berra' and consists of a mixture of barley and matra—(jao berra) or wheat and matra, or wheat, gram and matra. The wheat and barley having been trodden and winnowed at the 'Kalihan,' is ground in the hard stone Chakkī. The coarser parts of the pericarp are removed by sieving through a 'Chhanni,' the bran being known as 'Chokar,' but much of the pericarp remains in the flour which is eaten as a 'roti,' and the vitamin value of the seed does not appear to have been much impaired in the process of preparation of the flour.

*Lathyrus sativus*—styled matra and batra in Rewa, and variously known in India as 'kesari,' 'teora,' etc., has now to be considered.

The 'karya matra,' or black pea, which is the only variety of *L. sativus* grown in Rewa, is the large-seeded dark-coloured variety which grows on upland wheat land. If the seeds are small, the diminutive 'matrī' is applied to them—variation in colour yields another distinction, 'Bhura matra'—which is of slightly paler hue, and if the seeds are much flattened, it is called 'chipta matra'. All appear to be substantially the same stuff. 'Goliya matra' is the seed of another legume which I have not been able to identify but it is a close relative of our garden pea. It is grown in the Central Provinces in Damoh and Saugor, where it is called 'batra,' and there is used as a 'dhal' but not as a 'roti'. 'Bagalpurī matra' is the name given in Rewa to the small-seeded pale-coloured *Lathyrus sativus* grown on rice lands of the Gangetic plain, which is imported during famine years. It is not grown in Rewa, and is not seen there in normal years.

Matra is in Rewa the staff of life of the working classes at all times and, owing to its ability to grow in a soil which is too dry for the other 'rabi' cereals,—of the general population in years of scarcity. The labouring man likes it, he considers 'matra roti' to be 'filling at the price' and thinks its energy-yielding value to be considerable. An Abair (Rewa) kahawat exemplifies this view —

*Matra kī dhal men dal de hung,  
To reng chale Sambhar ke sing*

Translation 'Only put a dash of asafoetida in the dhal of matra, and even a dried sambhar's horn will (come to life and) walk.'

It is considered to be 'humble fairin' and a Brahmin patient will not readily own, 'coram publico,' to its extensive use in his own household. When questioned as to his diet preceding an attack of lathyrism, he will commonly give imaginary accounts of a sumptuous and varied fare whose accuracy one learned to discount by observing the grins on the faces of the listening audience, and from

Ankle clonus is marked and continues after the examining hand is withdrawn. The plantar reflex is very active and extensor in type. Much adductor spasm and limitation of abduction, with adductor tendon jerk, ditto in hamstring muscles, which are thrown into a clonic contraction by a tap on their tendons. No marked muscular wasting or trophic changes in legs. No anaesthesia or analgesia of lower extremities. There is no appreciable impairment of the functions of the hands and arms. He can grind the family grain, but a slight choreic movement of the arms was noticeable. Nystagmus was present, and the pupillary reaction to light was doubtful. He has a little difficulty in retaining urine when the bladder is full, but no incontinence of faeces. He claims sexual potency and has one child 3 or 4 years old.

The nystagmus and slight choreic uncertainty of the arm movements are unusual features in this case.

The history of his diet illustrates the state of affairs in a famine year (Plate XLIV, fig. 2).

*Case No. 25*—Lall Mun, male, age 30, Chatra, cultivator, he got ill 18 years ago in the famine year of 'Chhappan' in the month of Asar. He got fever, then the eruption of small-pox came out. Some 15 days after that, he fell down when at stool and got up to find himself lame. He recovered somewhat and was able to walk with a stick.

*Diet*—At that time, in a famine year as he was a poor man, he had to eat what he could get, chiefly matra, which was 12 seers per rupee and wheat 5 seers. Matha was obtainable from others but he had no cows of his own.

*Present condition*—A well nourished stalwart man, who walks with a marked equinus position of the feet. On the morning he was seen, he had walked five miles to attend the court at Sutna. When at rest, he stands on the flat of his feet. The toes drag as he walks, the foot is neither inverted nor everted. Knee jerks ++. Much spasticity of calf muscles and some spasm of adductor muscles. The plantar reflex is extensor, there is marked ankle clonus and the tendo achilles tendon reflex is very active. An adductor tendon jerk is elicited by tapping, the cremasteric, abdominal and epigastric reflexes present, no impairment of movements of upper extremities or cranial nerve functions. No *Romberg sign*, no anaesthesia or analgesia or lower limbs.

*Reactions to faradic current*—All muscles respond, the response of the *Tibialis Anticus* is very slight, and the response of all flexor muscles is strong (Plate XLIV, fig. 3).

At the other end of the scale of disablement are mild cases, of recent years in whom the degree of disability is so slight as almost to escape detection.

*Case No. 47*—B, female, age 14, caste Brahmani, daughter of C. B. of Kotar village. She got lame in Bhadon (August-September) of 1926. No fever or illness preceded its appearance, and she had been going daily to weed the 'kharif' crops during the rainy season, when a slight uncertainty of her gait was noticed by others. At this time she was eating the matra-barley berra of Kotar, in which lathyrus predominates, as a 'roti' in the morning, and in the evening, Bhat (boiled rice) with a 'dhal' of matra (lathyrus). With the 'roti' she was getting 'matha' (buttermilk), and mango 'panna' during the mango season, but no whole milk or ghee, and no 'chorai' or other green vegetable food.

*Present condition*—A comely well nourished adolescent girl who has not yet gone to her husband's house, menses normal.

*Gait*—A little jerky, with a linear foot track. She can heel and toe a line without any difficulty and can walk on her heels with the toes raised. There is no ataxia. The knee jerks are present and somewhat active and there is a little ankle clonus. The thighs can be fully abducted. The plantar reflex is inactive, but extensor in type, tapping the tendo achilles elicits a slight contraction of the calf muscles, and so also in the adductors. No tendon reflex in the hamstring muscles. No anaesthesia or analgesia in the lower extremities. No nystagmus or symptoms of cranial nerve impairment.

### EPIDEMIOLOGICAL

The following notes deal with the circumstances of Kotar, a village locally known as 'Lungra' or 'lame' Kotar, on account of the prevalence of lathyrism.

	Male	Female
Kotar	110	26
Abair	71	7
Kuan	53	12
TOTAL	234	45

The proportion in my cases was, therefore, about 5 males to 1 female Buchanan gave a proportion of 10 to 1 and whichever figure approaches more nearly to accuracy, there is no doubt that the sex incidence is very different, and that females are less liable to the disease

#### *Month of Onset*

Of 50 cases from whom reasonably reliable information could be obtained as to the month of onset, the following was the distribution by the months of the Sambat year —

(May)	Baisakh	1
(June)	Jeth	2
(July)	Asar	12
(August)	Sawan	9
(September)	Bhadon	9
(October)	Kuar	16
(November)	Kartik	1
	Agahan	} nil
	Pous	
	Magh	
	Phagan	
	Chait	

Among these cases, it was noticeable that of the 16 cases who assigned the onset to Kuar (October), nearly all were recent cases who connected the onset of their lameness with a preceding attack of fever (malaria ?) which prevails in the autumn. Famine year cases usually selected Asar (July) as the time of onset.

#### *Diet in Period Preceding Onset*

In all recent cases, and in most of the old cases, with the exception of those in whom, as might be expected, the lapse of years had effaced the memory of what they had been eating, a history of an exclusively lathyrus diet, or one in which lathyrus predominated, was elicited. A striking feature of such dietary histories was its monotony. A typical diet of a 'lugwa' suffering from lathyrism would be that during the three months preceding the onset of the disease, he had been eating twice a day, a 'roti' made from a flour of lathyrus, containing both pericarp and endosperm, taken with salt. This would be washed down with 'niatha' or buttermilk, or perhaps mango 'panna' during the mango season. No whole cow's milk, nor any ghee, nor a modicum of fresh vegetables,



In the neighbourhood of the village, in the fields which are irrigated by the inundation from the north, the crops chiefly of barley and lathyrus are rich and plentiful. A novel mode of manuring these fields prevails. The sweepings from the west end of the village are dumped on the lee side of a culvert and causeway which traverses the main line of drainage. When the floods come, and the water pours over this causeway the refuse is carried away and spread by these waters over the fields which they flood, and thus their fertility is effectively maintained. An inspection of the fields suggested a predominance of legumes, especially lathyrus, in the crops that they yield. An examination of the food grains used in the village and the accounts given by the villagers as to their diet confirm this. The heaps of 'rabī' grain crops given in barter in the bazar and in the bania's shops show a large and predominant proportion of lathyrus, which is of the large-seeded variety, the 'karya matra' of the wheat uplands of Rewa, with a small admixture of 'goliya matra,' another legume.

The commonest mixture is the 'jao berra,' of barley and lathyrus, the proportions varying, but in all cases lathyrus forms the larger portion. The mixture of lathyrus and wheat is less commonly seen, as the amount of wheat grown locally is small. As it is a valuable grain, for which there is a demand elsewhere, it is sifted out from the mixed crop and sold to raise money and the lathyrus is retained for consumption and for the wages of the field labourers. In both wheat and barley berra, there is a certain amount of gram.

In none of the numerous samples examined by me could the seeds of Akri (*Vicia sativa*) be detected, despite the plentitude of this weed in some of the fields.

Mango trees are numerous, and mango 'panna' is much drunk during the mango season, in May and June. Usually even the poorest obtain it, and it is used to wash down the dry and unpalatable 'roti' of matra, when no 'matha' or buttermilk is obtainable by them. Mahua trees are likewise abundant. The flowers are sun-dried and stored and parched for immediate consumption, forming a sweetmeat which is not unpalatable to European taste.

A wild plant yields a leafy vegetable known as 'Chorai,' which is gathered in Chait-Baisakh (April-May-June) by the Kachhiś and is bartered in the village by them. It is generally eaten during these months, at ten or twelve days' interval, but the poorer classes who have little food grain to spare for barter, cannot get it, and in a year of grain shortage, no one can afford to obtain it.

The staple diet of a better class Brahmin household, while the 'kharif' crop lasts, is, in the morning, a 'roti' made of barley and matra, the latter predominating, washed down with panna (mango decoction) or matha (buttermilk) according to the time of the year, and in the evening, rice, with a dhal of matra, cooked whole.

On this diet four mild cases of lathyrism occurred in 1926. The proportion of lathyrus in the diet varies in inverse ratio to the affluence of the family, and the poorer classes live for eight months of the year on an exclusive diet of lathyrus (nichela matra) and four months on Kodon, with a little dhan and some matra.

Families in comfortable circumstances can supplement this diet with some milk or ghee, and a little green vegetables, the poorer labouring classes have

shelter and sat down under a tree. On getting up he found himself unable to walk and had to be carried home, or case 44, who got lame in Bhadon 1925. He was at work in the fields when a rigor of fever, with cold and shivering seized him. He went home and lay up for four days. He got up and went outside to obey a call of nature and tried to jump a gap in a bund. He fell down, could not rise again and was brought home by his mother. In a very few cases prodromal symptoms of tingling and numbness, pins-and-needles feeling in the legs, or pain in the back, may have been noticed, but it seems clear that such are rare, or slight prior to the development of the syndrome.

In the 51 cases of which records were kept there was little variation in the signs and symptoms of a spastic diplegia.

In none of the cases were there any symptoms of mental impairment. Speech was normal, and the cranial nerves unaffected, except in one case, that of Chida, No. 27, in which there was nystagmus, in whom also an exceptional choreic movement of the upper limbs was noticeable. Impairment of the tactile and painful sensation in the lower limbs was always absent and so also was oedema or wasting of the muscles of the lower limbs. The superficial reflexes, cremasteric, abdominal, and epigastric, were often hard to elicit, and in some cases seemed absent. The knee jerks were always present and exaggerated, and sometimes a tap on the ligamentum patellæ would set up clonic spasms of the lower limbs. A tendon jerk could usually be elicited by tapping the adductor tendons, the tendo achilles, and the hamstring tendons. The plantar reflex, often hard to elicit in the horny soles of those who wear no shoes, was, when elicited, extensor in type in all but two cases with otherwise typical symptoms, in whom it was flexor in type. Ankle clonus was present in nearly all cases. In extreme cases the sphincter control may be lost at the onset but is regained later. Sexual potency is retained. *Romberg's sign* was always negative.

### *Electrical Reactions*

In such cases as an opportunity of testing the response of the leg muscles to the Faradic current was obtained, all the muscles responded to a fairly brisk stimulation at the motor points, the flexors more actively than the extensors. Arrangements for applying a galvanic current of sufficient voltage could not be extemporised under field conditions.

### *Gait*

The gait is very characteristic and has been very fully described by earlier observers. The writer found it most easily understood on the hypothesis that the disease is a lesion of the upper motor neurons, whereby the influence of the cerebral cortex in correlating the actions of different muscle groups has been disturbed, and the control of the postural reflex removed. This, which is normally a continuous act of extension of the lower limbs and spine, is disordered, the flexors overact and overcome the extensors, and the adductors overpower the abductors of the thigh.

Owing to the discouragement by the State of the growth of lathyrus, and the fact that the last few years have had good monsoon rains, and good grain is at present fairly plentiful, the 1925 and 1926 cases were comparatively few, and their symptoms light. Nevertheless the amount of lathyrus in the diet of this community is unusually large and to this fact coupled with the poverty and primitive mode of life, and the lack of the protective foodstuff in their diet, I would ascribe the unusually large number of cases of lathyrism in this village.

### ABAIR VILLAGE

Abair is a village some three miles east of Kotar. Its fields show the same predominance of legumes in their crops, particularly of lathyrus, and the village diet shows the same features, namely, a large proportion of lathyrus. The village population consists of 1,480 persons, 715 males and 765 females, and there are 71 males and 7 females affected with lathyrism, i.e., about 10 per cent of the male population and about 10 per cent of the female population.

### KUAN VILLAGE

Kuan village, in this neighbourhood, has a population of 393 males and 399 females. There are 53 males showing symptoms of lathyrism, and 12 females, i.e., 13.5 per cent of males and 3 per cent of females. All old cases.

### AMIRITI VILLAGE

Having noted the prevalence of lathyrism in Hindu communities who subsist on a diet in which lathyrus predominates and the protective food substances are lacking, it was now thought desirable to investigate, as a control, the prevalence or otherwise of lathyrism in a community using lathyrus in the diet and living under similar conditions, but with the addition to their diet of the 'protective food substances'. It was thought that a Mohammedan village should yield such information, and although such are few in Rewa, a scrutiny of the census returns showed that one such village existed, viz., Amiriti, a remote agricultural village in the Huzur Tahsil, containing 464 Mohammedans in a total population of 597. This village, was therefore, visited for me by Sub-Assistant Surgeon J. P. Shukla, and the following observations were recorded by him —

'The fields contain wheat, barley, gram and matra crops. The proportion of matra is about one-fourth to one-sixth of the total crop and several small fields of goliya matra were observed. A 'berra' of matra and other grains, in the form of 'roti,' is eaten here, as elsewhere, as the staple diet, with arhar and masoor dhals, vegetables are eaten once a fortnight. The village is on the bank of a river, and fish, caught by netting, is eaten every second day throughout the year. Flesh meat is also eaten, mutton, and goat's flesh, also flesh of deer, hares, tortoises, crabs, etc.

Little ghee is eaten although some families use it the custom being to make and sell the ghee to pay land rent.

There are 118 houses in the village, with a total population of 597, 204 Mohammedan males and 260 Mohammedan females. The rest are Kols and

hamstrings of both legs. There is much spasm of the adductors, the thighs cannot be separated, nor can the knee joints be extended much beyond a right angle. There is much wasting of the thigh and calf muscles. No analgesia or anaesthesia of lower limbs. Cremasteric reflex not elicited, abdominal and epigastric reflex not obtainable as contractures of the abdominal muscles keep the trunk in partial flexion, on account of which patient cannot lie on his back. There is no nystagmus, the tongue is protruded straight and the facial movements are complete. No tremors or stasis of the upper extremities.

*Case No. 22*—Serran, male, age 45, caste Chumar, pensioner at Sutna Lime Works, where he was a coal breaker. Has been lame for 15 years or so, being first attacked in the month of Asar. The history is that he first got a pain in the lumbar region and found that he was unable to walk over uneven ground. This gradually increased and by October he found that he needed two sticks to walk, when he came to the Lime Works as a coal breaker. Then pins-and-needles sensations began in his legs and he found himself unable to stand upright or to walk without falling, so he took to crawling. These feelings disappeared, but the inability to walk has remained. When at his worst, he had some trouble in retaining faeces and urine.

*Diet*—For three months before getting ill he was eating a bread of lathyrus and barley, the former predominating. The bread was prepared by making a flour of the pea and eating it without separating the husk. Buttermilk was drunk with it, but no ghee, milk or green vegetables were obtainable. He continued on the diet for six months after first getting lame.

*Present condition*—He crawls in seated position, with feet, buttocks and hands on the ground. His general health is fairly good, bowels regular, and no incontinence of urine and faeces. His lower limbs are wasted, the range of passive movements at the knee joints is to a point  $45^\circ$  short of full extension, owing to contracture of the hamstring muscles. The knee jerks are present but weak. The adductor muscles are contracted, and abduction of the thighs is very limited. An abductor jerk is elicited by tapping the abductor tendons. Ankle clonus is present. The plantar reflexes are very dull but an extensor response of the great toe was elicited in the right foot. Superficial reflexes epigastric, abdominal, cremasteric and gluteal not elicited. The scapular reflex was obtained. There is no anaesthesia or analgesia of the lower extremities. No tremors, no impairment of the movements of the hands and arms, nystagmus absent, facial and lingual movements unimpaired.

*Electrical Reactions*—With the Faradic current in both legs some contraction was obtained by stimulation of the motor points of the Tibialis anticus, Peroneus Longus, Adductors, and Quadriceps Extensor, Semimembranosus, Semitendinosus and Biceps.

No reaction in the Gastrocnemius and Soleus over the motor points but some movement was observed with the electrode over the Sciatic nerve below the gluteal fold.

This case illustrates the complete crippling produced by continuing with a lathyrus diet after the first symptoms have appeared. The electrical reactions indicate an upper motor neuron lesion even in an extreme case such as this (Plate XLIII, fig. 1).

*Case No. 27*, is an example of an extreme case, just short of the crawling stage.

Chida, male, age 30, caste Kol, occupation, was a hurwaha. He got lame in a year of famine, about 12 years ago when the crops failed and no grain was obtainable locally. Wheat was then 4 seers to the rupee and matra was 16 seers to the rupee and he had to eat this. He obtained it from Sutna Bazar and it was Bagalpur matra, imported grain. He could not then obtain any buttermilk, as he had no cows of his own and those who had buttermilk kept it for themselves. No green vegetable food was within his means and the only flavouring with this 'roti' was common salt. This matra diet was continued till Kuar (October-November) when he got Kodon, and Dhan and other food. After a day at earth-work on a bandh in Sawan (August-September) he was sleeping on a charpoy outside. A 'bad' or wind struck him and when he woke, he found himself unable to walk. He had no fever and no pain anywhere.

*Gait*—Uses two sticks as he drags his trembling legs along, with feet turned in and toes dragging along the ground. The knee jerks are much exaggerated and a tap on the patellar tendon sets up clonus in all the leg muscles, which has to be controlled by his hands. Marked spasms of the flexors and adductors, which can be overcome by gentle passive pressure.

and the population, especially the poorer classes, who suffer most from lathyrism, has been exposed to privation, malnutrition, and vitamin depletion, and are in a condition of grave nutritional instability, caused by a diet deficient in fat-soluble A

Some evidence in regard to the existence of an avitaminosis of A is afforded by certain facts that came to light regarding night blindness in association with the prevalence of lathyrism

McCollum (1925) states that 'this is a condition which seems related to a deficiency of fat-soluble A in the diet of man' He regards it as 'a specific syndrome of dietary origin' and suggests that 'a diet derived too largely from cereal products, tubers, and other foods having similar deficiencies, induces lowered vitality which becomes manifested, among other ways, in faulty vision'

My observations indicate that this condition is very common in Rewa

Major C H Smith, I M S, Superintendent of the medical department of Rewa State tells me that it is common in the jail, mostly in the hot weather months when vegetables from the jail garden are lacking and in this connection it is noteworthy that the fat element of the jail is derived from vegetable oils, not ghee

The Sub-Assistant Surgeon in charge of the Nagod State Dispensary tells me that he sees a number of cases in the hot weather months and that he connects its occurrence with a lack of fat in the diet

Mr Jardine, the Manager of the Sutna Lime Works, sees cases of this disorder of vision among his labour force and he first drew my attention to the belief in the efficacy of chuna leaves—'chuna ki bhaji,' as a remedy

For this complaint, known as Rataondhi, the traditional Indian remedy of a diet of goat's liver (a source of fat-soluble A), with a poultice of goat's liver to the eyes, is well-known

Ghee is also esteemed as a remedy and is used as such by those who can obtain it

A Brahmin cultivator expressed to me the unprompted opinion that those who eat ghee do not get Rataondhi and that the first milk of a newly calved cow is also efficacious as a cure

'Chuna ki bhaji,' the green prunings of the young chuna plant is, however, the most universal remedy among the vegetarian poorer classes Thus in the family of a lathyrism case No 51, the story was told me that the young wife of the patient had, in August of the year in which the husband developed lathyrism, suffered for six days from night blindness Her old mother-in-law produced some dried 'chuna ki bhaji' which was kept in the house, soaked it in water, put it up on the roof of the house overnight, and fed the patient on this, who found herself cured in two days

It is significant that in one family the man developed lathyrism and the wife night blindness in the same month and on the same diet

That the association is not fortuitous is suggested by the observation that in Kotar village where, as we have seen, 12 per cent of the male population are afflicted with lathyrism, night blindness is extraordinarily prevalent

among its inhabitants and those of adjoining villages. This area well illustrates the conditions under which lathyrism prevails, and as such may be considered in detail.

### 'LUNGRA KOTAR'

The village is some nine miles east of Jetwar station on the G I P Railway, with which a cold weather cart track connects it. It is situated on a hillock, and is grouped round a ruined Baghel fort, which is said to have been built by Maharaj Bhao Singh in 1675, and has for many generations been abandoned. The environs of the village show evidence of departed prosperity in the shape of numerous large tanks, now used for washing clothes and bathing, which doubtless served the needs of a population considerably greater than that which now utilises them. The village is within a mile or two of the Tons river and surrounding the hillock on which it is situated, is low-lying ground, along which the drainage of high ground to the north of the village runs to join the river. This low ground is intensively cultivated by Kachhis, but the green crop which they grow by copious irrigation with water raised by 'dhenkuris,' is not one of the edible vegetables, but of tobacco, which is grown for export, as its flavour is much esteemed locally.

Cattle are numerous, but as is the practice in Rewa, most of the exiguous yield of milk is utilised for the manufacture of ghee, which is sold to raise money to pay the taxes and only the better class families consume a portion of the dairy products of their own animals.

The people are all agriculturists, and there are no industries of any sort. The 'Kachhis' export dried tobacco, and ghee, and a little superfluous food grain is exported and these form the only outside sources of income of the village. There is little circulation of money, and in the weekly bazar and in the bania's shops the peasants purchase their simple needs of salt, gur, and the cotton fabrics used for clothing, by bartering an equivalent value of the food grains of their own crops. The 'kharif' crops are rice and Kodon, chiefly the former, and they appear to be somewhat scanty. The people of the poorer classes who are dependant on daily wages or on 'khawar,' a payment in kind, tell me that they live from December to March on the grain of the 'kharif' harvest, viz, Kodon and rice, and for the remaining eight months of the year on the cheaper grains of the 'rabi' crops. These grains are wheat, barley, matra, the pulses, masoor, etc, and a little gram.

On nearing the village and crossing the fields, one is struck by the preponderance of legumes in the growing crops.

There are a few fields of wheat and matra, of poor and stunted growth, and barley and matra is more plentiful. There are many fields of masoor and a little gram, and in all these there is a superabundance of *Lathyrus sativus*, which is also grown by itself or mixed with the pea called 'goliya matra,' which is more common here than what one sees elsewhere.

In the low-lying fields in which 'kharif' crops of rice are raised, there is an unusual amount of Akri (*Vicia sativa*).

As is the custom elsewhere, this growth is assiduously weeded out and fed to the cattle.

privation, on the inadequate 'rabi' crop of 1920-21, supplemented by cheap imported grains

It has been shown that in a normal year, no importation of grain occurs, and that the 'Bagalpurī matra' whose germination he suggests to be the cause of the disease, is never used. He suggests that the indigenous vetch is perhaps non-poisonous, but of the 13 recent cases seen by me all had been eating locally grown matra and of the eight samples of such grain that were obtainable, all were of the large-seeded variety, the 'karya matra' of the wheat uplands of Rewa.

From this it would appear that the locally grown matra is as potent to produce lathyrism as is the imported 'Bagalpurī matra' whose consumption in a famine year has earned it an undeservedly evil reputation in Rewa,—a disrepute probably due to the circumstances of its use rather than to any inherent and special toxicity.

He noted that the majority of cases seen by him gave the month of July as the time of onset, and he suggested that this indicated the possibility that germination of the grain occurs during the rains, which produces the poisonous amine to the ingestion of which he considers the symptoms of lathyrism are due.

Buchanan (1904) paras 52 and 64 to 67 of his report, has shown that three months of lathyrus diet at any time of the year will determine the onset of lathyrism, and it would appear that the facts which he cites afford a credible explanation of the appearance of the cases in July when three months of preponderating lathyrus diet since the harvesting of the 'rabi' crops in April, had been accomplished, without assuming germination and a poisonous amine to be the explanation.

The possibility of the occurrence of such germination was, however, carefully investigated. In none of the specimens of *Lathyrus sativus* which had formed the staple diet of eight cases before the onset of their disease, was any sign of germination detectable, and this was confirmed by the Director of the Institute of Plant Industry Indore, A. Howard, Esq., C. I. R., to whom samples were sent for examination.

It does not appear likely that such can occur. The grain is harvested in the hot dry month of April. It is stored in the houses in a large sun-baked earthen jar called the Kutuli.

Grain which is to be used for seed is mixed with wood ashes to prevent the inroads of weevils, while that required for use is kept in the Kutuli and taken out and spread in the sun at frequent intervals to keep off weevils. It is unhusked, and the pericarp is thick and resistant and unlikely to permit of the germ being activated by an increase of atmospheric humidity.

I was informed by all whom I questioned, that only such grain as has been soaked in water will germinate, and that if it does, it is rejected as unfit for food.

No field evidence can therefore be discovered which suggests that an imported grain is the offender or that germination of the grain occurs, while the July incidence seems more credibly explained by the completion in that month of a period of three months' diet of lathyrus rather than by the occurrence of a hypothetical germination.

none of the former, and little of the latter, except for such small amount of chuna shoots, 'chuna ki bhaji' which they obtain in the cold weather. Flesh meat is hardly ever eaten, even by those castes who indulge in it, except on the rare events on which it is obtainable as game.

According to the village school master, malarial fever is very prevalent in October, as might be expected from the amount of the low-lying land which surrounds the village.

The population of the village in the last census was 1,774, all Hindus except three, who were Mohammedans. There were 369 houses, 856 Hindu males and 918 Hindu females.

A rough census of the number of cases of lathyrism in the village was obtained by summoning a prominent man from each Moholla and getting him to name those in his quarter afflicted with lameness. The figures thus obtained were 110 males and 26 females, total 136.

On these figures it would appear that 7.6 per cent of the total population, or 12.8 per cent of the male population, and 2.8 per cent of the female population are affected with lathyrism, and it is probable that these numbers are an underestimate.

It appears, therefore, that the description 'lame Kotar' currently applied to this village, is justified by the condition of a population, 12.8 per cent of whose adult males are crippled by lathyrism.

On the supposition that lathyrus diet is the primary cause of this calamity, the state of affairs in this village is readily explainable. From the foregoing notes as to crops and diet, it will be seen that the population lives very largely on a lathyrus diet, which is the predominating food grain in this area. A local *kaliawat* expresses this dependance on lathyrus in rhyme —

*'Matra ki roti, matra ki dhal,  
Matra hi pati rakhan har'*

which may be translated as follows —

'Bread of matra and matra as dhal  
Matra indeed is the guardian of all'

Possibly the barley-lathyrus 'berra' which they use is a more dangerous diet than the wheat-lathyrus more common in other Mouzas of the Rewan 'Uparihar' and the lack of variety and the comparative shortage of the 'kharif' crops of Kotar may also be an unfavourable factor.

The villagers say that in former years they grew much more matra than they do now, and that this was the cause of the excessive lameness and of the epithet 'lame Kotar,' and they recall that in the famine years, to which all the older and more seriously affected cases refer the onset of their complaint, nothing but matra grew.

Under present-day conditions, a small annual crop of mild cases is occurring. Nine cases were reported to have occurred in 1926, of these 6 cases were seen and diagnosed as lathyrism. One was absent from the village, one was not a case of lathyrism but of injury, and a third was of doubtful causation and may have been syphilitic, 5 cases of 1925 were seen.



seeds of Akri may be found in a handful of wheat from the bottom of a heap. The large-grained 'Karya matra,' the cheap grain which is fed to 'lugwas' and whose consumption causes lathyrism, ordinarily contains no Akri.

The only crop in which a very small percentage of Akri may be found is a 'berra' produced in a somewhat moist field which in the previous year had been sown with wheat and matra, etc., and for some reason is being allowed to lie fallow under a self-sown, unweeded crop, consisting chiefly of matra and a little wheat, with some wild vetch in places.

Such a crop is given to the 'lugwas' and may admittedly contain 'one pice in the rupee' of Akri, but such slovenly methods of farming are very rare, I only saw one such field in four months' wanderings.

None of the many samples of matra in current use which I collected contained any Akri seeds, which are easily distinguished from the large and characteristic seeds of the upland wheat lands, although it may be more difficult to distinguish them from the smaller seeds of the grain grown on rice lands. Six specimens of grain which had been eaten by lathyrism cases were sent to

	SAMPLE I	SAMPLE II	SAMPLE III	SAMPLE IV	SAMPLE V	SAMPLE VI
Barley	250	18	293	3	2	28
Wheat	28	891	36	807	32	6
Rice	13	1	3			4
Linseed	9	4	0			1
Gram lentils, etc	22	9	7	16	60	7
Kesari	489	657	303	497	657	864
Akta	54	0	15	0	0	0
Earth	A great deal	Very little	None	None	None	A great deal
Weevils *	Weevilled but no live weevils	Many live weevils	Few weevils	Very weevilled, many live weevils	Very few weevils	No weevils

\* The weevils which infest matra were sent to Mr Bambriggs Fletcher, Imperial Entomologist, Pusa, for favour of examination.

He reported 'that they are not real weevils, but are a species of *Bruchus* (Bruchidae) of which several species attack pulses in India.' He advises that they occur practically throughout the world in various species of peas and beans and that it is therefore unlikely that they are concerned in cases of lathyrism.'

chamars who are 'lugwas' of the cultivators. Five persons are reported to be lame, three females and two males. Two Mohammedan females have been lame since birth or early childhood and a Kol woman has a fractured leg. Of the 2 male cases, one, a Mohammedan, became lame 12 years ago after smallpox. He was not examined, but his gait with widely spread foot-track was not that of lathyrism. The second has a sinus in connection with the knee joint. There are no old lathyrism cases of the famine year in this village. In 1897 when the food grains were short, they supplemented their diet with fish and game, and the superfluous cattle were probably killed off and eaten' (although this would not be expressly admitted in a Hindu State).

The 'lugwas' in ordinary years share in the flesh diet of their masters, and none of them have ever developed lathyrism even in famine years.

'The state of affairs in this village as compared with Hindu villages is significant. Matra forms as large a proportion of their cereal crops as it does in the Hindu villages, but it would, in Rewa, be almost impossible to find a Hindu village of this size which is free from some cases of lathyrism. Its immunity seems to suggest that a better balanced diet with a higher vitamin content enables a population to use lathyrus in considerable amount without harm accruing.'

### DIET AND LATHYRISM

It will be seen that nothing has come to light in this investigation which casts any doubt on the age-long experience of the human race that a lathyrus diet produces lathyrism. The kahawat which heads this report was related to the writer by an intelligent peasant in a Nagod village and embodies their experience of the results of eating matra.

Ample confirmation was also obtained of Buchanan's dictum that a three months' diet of one-third to one-half of lathyrus is dangerous.

This, however, is not the whole story, for many there be who eat lathyrus, who do not get lathyrism and it seems from these investigations that certain conditions of nutritional instability are important, and perhaps essential factors in the production of the disease.

It has been shown that in the diet of the Rewan peasantry the green leafy vegetables and dairy products are conspicuous by their absence. During the dry hot weather months green vegetable food for man is lacking and the cattle are on starvation diet, meagrely fed on the few pickings they can obtain on the pasture lands, supplemented by dried fodder, the straw of the 'rabi' crops. What milk they yield is presumably deficient in A. The supplies of vitamin A in the human diet, ordinarily small, are in this period especially in defect, and these are the months from April to September when the 'rabi' crops are the staple food, and the poorer classes, and, in a famine year all but the richest, live on a lathyrus diet. July is the month when a period of three months of lathyrus diet is accomplished, and the lathyrism cases begin to appear.

Moreover, in a year of deficient rainfall and famine, when such cases are numerous, the 'kharif' crops normally varied in kind and plentiful in quantity, upon which the poorer classes depend for their nourishment, have been inadequate,

only those whose circumstances of life approximate to famine conditions acquire lathyrism and the striking feature of a pre-lathyrism diet, apart from the preponderance of lathyrus, is the absence from it of the protective food substances of McCollum. The notes on Kotar village show that where one of the recognised effects of a diet which lacks in fat-soluble A viz night blindness, is very prevalent, lathyrism is rife, whereas the 'control' Mohammedan village of Amiriti, also using lathyrus as a foodstuff, but adding to its diet other articles as eggs, fowls, fish and flesh meat, is entirely free from lathyrism. These indications seem to point to the possibility that lathyrism is allied to the deficiency diseases in the mechanism of its causation.

Further examination of the available data seems to show that such a hypothesis supplies some explanation of the epidemiological and ætiological facts already noted. In regard to the greater incidence in the years of greatest physical activity, the ages 10 to 20, and 20 to 30, an analogy with the deficiency diseases, beri-beri and pellagra may be noted, the incidence of which is highest among those whose work is hardest (McCarrison, 1921). The influence of excessive physical exertion in determining the attack of deficiency diseases has been noted by McCarrison who records that 'the onset of human beri-beri is often rendered acute, or that of hunger œdema sudden by excessive physical exertion' and he quotes others in support of the view that 'exposure to cold favours the onset of scurvy, malnutritional œdemas and pellagra'. As we have seen, exertion and wettings are often the immediate antecedents of lathyrism.

In regard to sex incidence, which is from 5 to 10 times greater in males than in females, apparently only an essential sex difference can explain it, for the women share equally in the work of the fields and their diet differs in no way from that of the men.

McCarrison notes a different sex incidence in the deficiency diseases, beri-beri and war œdema, and in experimental polyneuritis columbarum and attributes this in part to 'different metabolism and endocrine action in the two sexes'. No other explanation seems applicable to the markedly different sex incidence in lathyrism and the analogy is perhaps significant.

It has been shown in these notes that a common antecedent of lathyrism is an attack of malarial fever (which is often treated by ten days' starvation, 'Upas') or by dysentery, smallpox, or pneumonia.

While such may be regarded as conditions tending to lowering of the vitality, and as such sufficiently explained, from the point of view of dietary studies, they may perhaps be regarded as periods of vitamin depletion which would hasten the appearance of a deficiency disease. The six months of starvation after an insufficient 'kharif' crop which precedes the mass outbreak of lathyrism in a famine year, may perhaps be similarly construed.

In regard to a preponderance of the legume *Lathyrus sativus* in the diet of a community suffering from lathyrism and its bearing on the subject under discussion, we may recall views of recent workers in dietetics.

McCollum (1923) states that 'the protein molecule is a chain-like structure of amino acids' and points out that 'the nutritive value of a protein depends on its yield of the indispensable amino acids and the extent to which their

This is embodied in a local kahawat, a jibe against Kotar, which runs as follows —

‘*Ekar andhar, ekar lul,  
Ekar chalc matkawat kul,  
Ye dekho Kotar ke shul*’

‘One is blind, another halt, and another lame with swaying hips, behold the troubles of Kotar!’

Inquiries showed that the particular form of ‘Andhar’ was night blindness, while the tilting and wriggling movement of the pelvis in lathyrism—‘matkawat kul’—is considered a diagnostic feature of that disease

My case notes show that in the diet of lathyrism cases in particular, as in the diet of the population in general, there is a conspicuous absence of the leafy vegetables, and of whole milk, ghee or dairy products containing butter fat, which are believed to be the most plentiful sources in a well balanced vegetarian diet of fat soluble A, while in regard to Kotar it is seen that this community which suffers severely from lathyrism, also suffers from night blindness, an evidence of the lack of fat-soluble A in its diet. With this may be contrasted the state of affairs in the ‘control’ village of Amriti, whose inhabitants grow and eat as much matra as the average Hindu villagers do, but being Mohammedans they supplement their cereal ration with fish and flesh meat and substances containing a modicum of fat-soluble A. This village has no lathyrism cases

In Sind, which the writer subsequently visited on another inquiry, it appears that *Lathyrus sativus* is grown and eaten, but cases of lathyrism—known as ‘matar mandai’ are very rare, and occur under exceptional conditions of scarcity. The Sind villager grows and eats *Lathyrus sativus*, but he also keeps herds of fine upstanding cattle and buffaloes, which give a plentiful yield of milk, which is consumed by the owners, and they also grow and eat green vegetables in considerable quantities. The conditions which, with a lathyrus diet, are in Rewa associated with the occurrence of lathyrism are absent in Sind. I am indebted to Dr Shiveshwarkar, Assistant Director of Public Health, Sind Registration District, for the opportunity of perusing his report on an outbreak of scurvy in a Sind jail where, in addition to the lack of vitamin C in the diet, the substitution of lathyrus dhal for the other more expensive legumes from which it had been previously prepared, was associated with the appearance of symptoms of lathyrism. This observation would also appear to mark an association of lathyrism with nutritional instability and avitaminosis, in this case of C.

### ACTON'S AMINE THEORY

It is now for consideration what bearing the observations have on the two theories of causation which it has been the work of the writer to investigate in the field

In regard to Acton's theory of the formation of a poisonous amine by germination, it may be noted that his investigations (Acton, 1922) were made in August 1921, i.e. in a period following the year 1920 when the rains and the ‘kharif’ crops had failed and the people were subsisting after eleven months of

Further experimental work, reproducing as far as possible in animals the pre-lathyrism avitaminosis as seen in men, would, therefore, seem to be desirable. If successful, the results of such work would yield deductions of considerable practical value.

Pending such confirmation, some practical preventive inferences seem, even now, to be warranted. As Acton has pointed out (Acton, 1922), the ultimate causes of lathyrism are economic. Its prevention turns on administrative improvements aimed at reducing poverty, providing a better balanced diet, and preventing the exclusive use of *Lathyrus* in years of deficient rainfall.

In Rewa, irrigation would prevent this dependence on rainfall, but as to whether a system of 'flow' irrigation, utilising the waters of the river Sone, is a practical engineering proposition and one which is within the financial resources of the State, the writer is not competent to offer any opinion.

'Lift' irrigation from wells, using a water lift operated by bullocks—is a possibility which would similarly require technical consideration. Its introduction would presumably require capital for initial expenditure and maintenance, which the 'rayat' has not got, and the State would presumably have to finance it by loans.

An obvious requirement is an increase in the village roads and cart tracks.

This would make it worth while for the villager to grow more and better grain, which he could get away, and sell, and thus provide himself with better food and with some financial reserves to tide over a period of stress. It would also enable the State to supply him with a grain not liable to produce lathyrism, when, in the absence of irrigation, famine relief measures are required in a year following a bad monsoon.

If the writer's views are well founded that avitaminosis due to lack of the use of dairy products and green vegetables, is an essential factor in the production of lathyrism, then measures aimed at increasing the yield of milk by improving the breed of cattle and at encouraging the growth and use of vegetables, would be valuable in its prevention.

The prohibition of the cultivation of *Lathyrus sativus* has been tried by State administrators in Bundelkhand, and Baghelkhand, following the example of European rulers of the 17th century (Stockman, 1917).

It has been shown that this vetch is normally sown as a mixture with wheat or barley, etc., as an insurance against famine, and effective prohibition would leave the cultivator with nothing to eat in dry year when his wheat failed. The order is usually evaded by a mixed sowing in which, it is claimed, the *Lathyrus* is self-sown, as indeed much of it undoubtedly is.

Furthermore, this hardy and universally cultivated legume, some of which is present in every wheat-field in Rewa, doubtless serves a useful function in conserving the nitrogen of the soil in fields which are not manured and are continuously cropped, and it almost certainly serves a useful purpose for this reason if no other.

If not used in undue proportions, it is a valuable food crop and in the opinion of the writer, efforts should rather be made to obviate, by the measures

## AKTA CONTAMINATION THEORY

It will be recalled that Anderson, Howard and Simonsen (1925) observed that in 30 specimens of Kesari obtained from different parts of India, the only foreign constituent common to all was the narrow leaved vetch, *Vicia sativa* var *angustifolia*, known in the vernacular as 'Akta'

Their chemical investigations showed that the seeds of *L. sativus* contained no alkaloid but the seeds of *V. sativa* var *angustifolia* were found to contain traces of two bases showing alkaloidal properties, viz, vicine and divicine, and a cyanogenic glucoside, vicianin. Divicine produced by inoculation of guinea-pigs a characteristic fatal disease, and a series of animal experiments indicated that in ducks and monkeys fed on Akta the majority showed a definite syndrome somewhat resembling human lathyrism, although by no means entirely similar to it—symptoms which were absent in 'controls' fed only on Kesari.

The possibility that lathyrism is due to the presence in commercial Kesari of the seeds of this contaminating weed was, therefore, examined in great detail in the course of this investigation.

This weed which is known in Rewa as 'Akri' is a wild vetch which grows in moist soils. It is to be found in most fields which surround a tank. Some may be found in a moist corner of a wheat field at its lower end where a bāndh has been thrown up to retain in it the monsoon rains. It is sometimes fairly plentiful in 'dumat' fields which grow rice during the 'kharif,' and wheat, etc., during the 'rabi' season.

It is a true weed, and it is never seen in the drills in which the seed has been sown through the 'nali,' but in the soil between the furrows.

If the seed were a constantly present contamination of the harvested crop, it would be found also in the sowings, as no special pains are taken to purify the seed which is sown, which is identical with that consumed.

Its absence from the drills, which I was at pains to verify by a careful study of a large number of growing wheat fields, shows its absence from the sown seed and seems to afford a reasonable presumption of its absence from the crop as it is eaten.

Everywhere the villagers were emphatic that it is bitter unpalatable stuff which is never eaten by man and that it is always weeded out and used as cattle fodder. They say that if a field contains an appreciable quantity of it, it must be carefully weeded out, else it will damage the crop and reduce its yield.

During February, I was everywhere able to see this work in progress and to mark the bundles of Akri being brought home as cattle fodder (Plate XLVI, figs 8 and 9).

The villagers point out that the Akri pods ripen before the matra pods and that they burst and scatter their seeds on the ground before the matra is harvested, and that little or none of the Akri which has not already been gathered for cattle fodder is mixed with a 'berra' or a matra harvest. Matra is usually grown as a mixed crop—a berra, and this mixed crop is usually passed through a sieve to separate the more valuable wheat or barley from the cheaper matra. The matra seeds being the larger, are retained by the mesh through which the wheat passes, and this explains why a very occasional seed of the minute

EXPLANATION OF PLATE XLIII

- Fig 1 Sepaiya, a crawler  
„ 2 Chida, much adductor spasm and spastic equinus

Mr Howard for examination as to the presence in them of Akri contamination. In specimen No 1, which was the leavings at the bottom of a grain bin, to the bottom of which the small seeds of Akta and grains of earth, and other debris had found their way, 54 seeds suspected to be those of Akri were separated from a total of 489 of Kesari, not including seeds of other food grains. The average weight of Kesari seeds to Akta seeds is about 5.5 to 1 and the proportions by weight of Kesari and Akta were therefore about 50 to 1, while the percentage by weight of Akta in the mixture, which also contained barley and other food grains, was still lower. In specimen No 3, also one of leavings, the proportion by weight of Akta to Kesari was about 111 to 1 and was considerably less in the total mixture.

It has been remarked that the weed is more abundant in 'dumat' fields, which grow rice in the 'kharif' and are more moist than the wheat lands. Were this contamination the cause of lathyrism, one would expect the disease, with the weed, to be more common in rice lands than in wheat lands. In Rewa I could find no evidence that this is the case, nor does it appear that the disease is more prevalent in the rice lands of the Gangetic plain than it is in the upland wheat lands of Rewa, where the weed is rare and the disease is common.

The villagers are emphatic in rejecting the suggestion that Akri is ever mixed with matra or that it is eaten in any appreciable quantity, and the opinion of those who sow, grow, weed, reap, harvest, grind, bake, and eat their own grain is worthy of credence.

They clench the matter by pointing out that 'Akri grows only in moist fields and that in a famine year when lathyrism cases occur in great numbers, the fields are all dry and no Akri grows. how, then can it produce lathyrism'?

Taking everything into consideration, therefore, I can find no confirmation of the theory that lathyrism is caused by a contamination of *Lathyrus sativus* by the weed of *Vicia sativa*.

### DISCUSSION

Having failed to find evidence that lathyrism is due to an amine formed by germination or to contamination of the grain by a poisonous weed, it remains to discuss what other possibilities of causation are suggested by this investigation.

Lathyrism is pre-eminently a famine year phenomenon, it is one of the pains and penalties of poverty and malnutrition, and the mechanism of its production is as follows —

In a year when monsoon rains fail, the 'kharif' crops on which the poorer classes depend for a varied and cheap variety of cereals are in defect, and the people are now ill-fed and half-starved. The wheat and barley of the 'rabi' crops fail to germinate in the dry soil, only the lathyrus grows, which is harvested in April, and the bulk of the population has to live chiefly on that and on such imported grains as they can obtain, of which lathyrus, being the cheapest is the most used.

Three months of such a diet brings on an abundant crop of lathyrism cases, beginning in July and continuing until the resumption of a better balanced diet in September and October when the 'kharif' crops become available if the succeeding monsoon rains have been more bountiful. In a year of normal rainfall



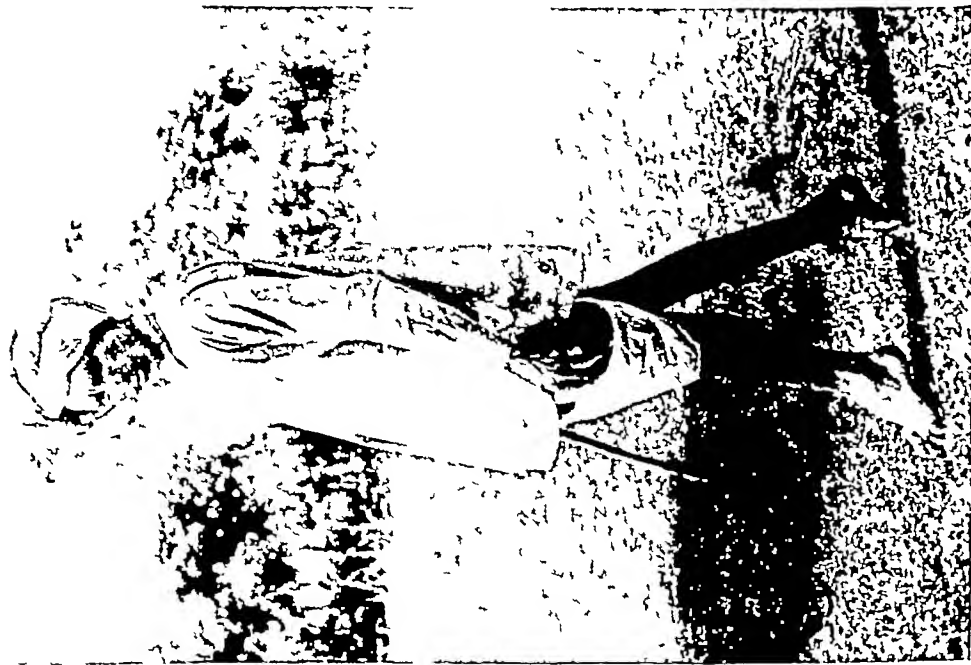


Fig. 3

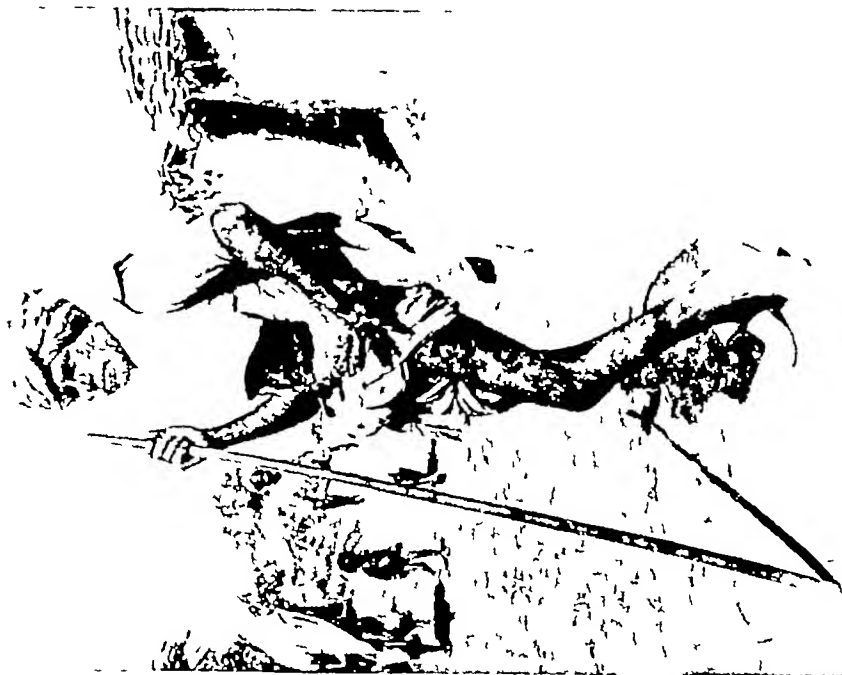


Fig. 4

proportions correspond to those existing in the body proteins into which they are transformed' In regard to legume proteins, he is of the opinion that 'they have peculiarities in their composition which make them of relatively low value in nutrition when they serve as the sole source of protein in the diet, or when they are combined with the more important foodstuffs'

Ellis and Macleod (1922) state that 'pea protein is an unsatisfactory diet' and they deduce from animal experiment that 'pea protein contains something which is injurious in large amounts over long periods'

Elsewhere it is stated that 'legumes lack fat-soluble A, and the mineral contents of a well-balanced diet'

In regard to the influence of an unbalanced diet in determining the onset of the deficiency diseases, McCollum quoting Appleton and others, in regard to a community living in Labrador in a state of extreme nutritional instability, writes 'small deviations in the constituents of a diet determine whether beri-beri, scurvy or ophthalmia appear'

### CONCLUSIONS AND DEDUCTIONS

We have seen that—

1 When the legume, *Lathyrus sativus*, predominates in the diet, lathyrism may result

2 When the general population is suffering from starvation and avitaminosis in a famine year, lathyrism is more common than in normal years, when these conditions are absent

3 A community suffering from lathyrism is also in a state of marked nutritional instability due to lack of the protective food substances, notably those containing fat-soluble A

4 In an area in which lathyrism is particularly prevalent, the deficiency disease, 'night blindness,' is also notoriously prevalent

5 In a Mohammedan village using as much *Lathyrus sativus* as their Hindu neighbours but supplementing their diet by substances which tend to restore the dietary balance, lathyrism is unknown

6 There are some significant resemblances in the ætiology of the deficiency diseases and lathyrism

It may tentatively be suggested for experimental verification that lathyrism may be to some extent a deficiency disease which is produced in persons living in a state of nutritional instability on a diet noticeably lacking in vitamin A, by a prolonged ingestion of a legume, the amino acids of whose proteins are unsuitable as a diet and perhaps specially harmful, which is itself deficient in fat-soluble A

In regard to such experimental work it may be noted that while epidemiological observations point to a prolonged period of avitaminosis being an essential factor in the production of lathyrism, experimental workers, Stockman (1917), Anderson, Howard and Simonsen (1925), were at some pains to eliminate as far as possible, the influence of avitaminosis on their experimental animals

The observations cited in this report suggest that this failure to reproduce the conditions which attend the onset of lathyrism in man may have been the cause of their lack of success in reproducing the disease in animals

EXPLANATION OF PLATE XLV

- Fig 5 Adductor spasm and spastic equinus  
„ 6 Cross legged scissor gait due to adductor spasm

above outlined, its involuntary misuse by over-use, in preference to endeavouring to suppress it by legislation which cannot in the nature of things, be obeyed

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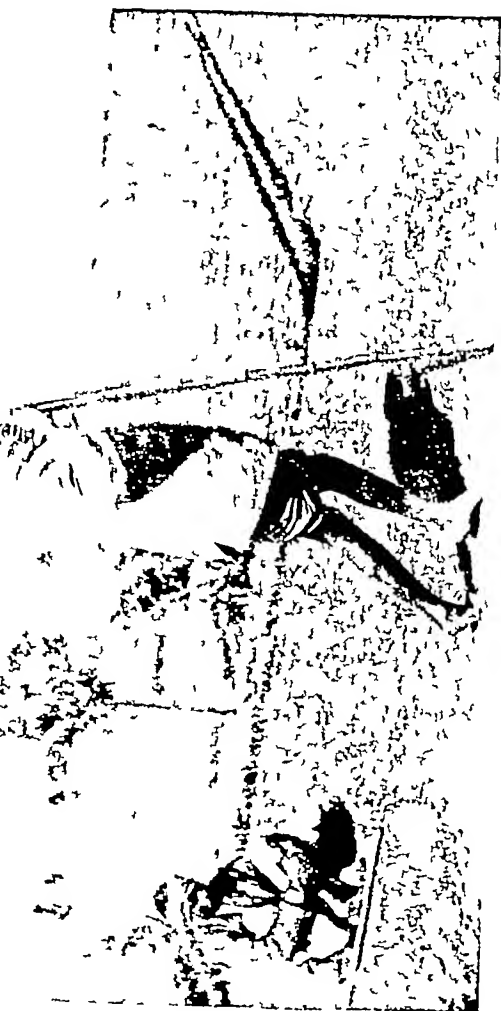


Fig 7



Fig 8



PLATE XLIII.



Fig 1



Fig. 2.



#### EXPLANATION OF PLATE XLIV

- Fig 3 Lall Mun, marked spastic equinus  
„ 4 Spastic equinus and adductor spasm



NNN tube was inoculated, but if there was sufficient a drop of material was placed into each of two or three tubes. The tubes were kept for various periods, from 4 to 28 days, in an incubator at about 25°C. When the cultures were ready to be inoculated into mice, a drop from each tube was examined to ascertain that the culture was 'positive' and that it was not contaminated. A note was usually made as to whether the culture was scanty, medium or rich, the number of flagellates usually varied according to the age of the culture. The surface of the solid part of the medium was scraped with a platinum loop and the whole fluid contents of the tube injected into the peritoneal cavity of the mouse. If there were two or more culture tubes from one patient, these were not injected at the same time but at intervals of about a week.

*Examination of the mice* — The mice were kept for periods from three to four months, after which they were killed and examined. The dead mouse was immersed in tincture of iodine, it was stretched on a board and opened with the aid of two sets of sterile scissors and forceps, the first set being used for opening the abdomen and the second set for snipping off portions of the organs to be examined. The contamination rate in Calcutta is very high and I found it better to add portions of the organs, spleen and liver, directly to the NNN medium than to crush them up with the aid of broken glass, as suggested by Christophers, Shortt and Barraud (1926). Four tubes were inoculated from each mouse and smears made from the liver and spleen. The smears were examined immediately for leishmania, and the NNN tubes after 14 days' incubation at 25°C.

#### THE RESULTS OBTAINED

Twenty-two of the mice died within 3 months. In six instances all the cultures were contaminated, in three of these the smears were 'positive,' but in the other three the smears were 'negative' and in these cases the mice have had to be considered as not infected. Altogether forty-four mice were killed and examined, of these 14 had been inoculated with cultures of 6 days or less, 18 with cultures more than 6 days but less than 12 days, and 12 with cultures of more than 12 days of age. The accompanying table gives details of the 15 mice that became infected.

The mice that were examined can be arranged into three groups, as follows —

14 mice injected with culture less than 6 days old, 3 infected

18 mice injected with culture less than 12 days but more than 6 days old,  
7 infected

12 mice injected with culture more than 12 days old, 5 infected

In the last group three 28-day cultures did not give rise to infection

Thus, in order to infect one mouse 4.67, 2.57 or 2.40 mice, according to the age, of the culture used, had to be inoculated.

#### CONCLUSIONS

There is only one absolutely definite conclusion that can be drawn from these results, namely, that the early flagellate forms will give rise to infection in mice when injected intraperitoneally. Only tentative conclusions can be drawn from



Fig 5.



Fig 6



EXPLANATION OF PLATE XLVI

Fig 7 Progression with bent knees due to flexor spasm

Figs 8 and 9 Akri gatherers

Table showing the results of the experiment designed to prevent Stone-in-the-bladder in rats

Lab No of Rat	Sex	Initial body-weight Grms	Final body-weight Grms	Days under experiment	Cystitis	Hæmaturia	Stone-in-the-bladder	Weight of calculi Mgrms	Dilated ureter	Hydro- or pyo-nephrosis	Stone-in-ureter	Stone-in-kidney	Cause of death
18 ANIMALS RECEIVING OATMEAL DIET AND WATER													
685	M	50	52	66	no	no	no		no	no	no	no	Asthma
686	M	51	72	91	"	"	2	58	"	"	"	"	Hæmorrhagic enteritis and stone
687	M	51	85	107	"	"	no		"	"	"	"	Pneumonia
688	F	50	90	98	yes	"	gravel		"	"	"	"	Asthma, cystitis and gravel
689	F	48	77	112	"	"	"		"	"	"	"	Asthma, cystitis and gravel
690	F	46	65	130	"	"	"	25	"	"	"	"	Asthma, cystitis and gravel
691	M	52	75	89	no	"	no		"	"	"	"	Pneumonia
692	M	50	69	94	yes	"	gravel	92	"	"	"	"	Asthma cystitis and gravel
693	M	54	84	77	no	"	no		"	"	"	"	Asthma
694	F	50	102	134		"	"		"	"	"	"	Pneumonia
695	F	48	74	147	yes	"	24	99	"	"	"	"	Asthma, cystitis and stone
696	F	46	67	148		"	no		"	"	"	"	Asthma, cystitis
697	M	51	90	150	no	yes	"		"	"	"	"	Pernicious anemia
698	M	55	95	150	yes	"	gravel	24	"	"	"	"	Pernicious anemia, gravel
699	M	58	148	149	no	"	Fibrinous mass		"	"	"	"	Pernicious anemia
700	F	50	46	136	yes	no	1 and gravel	24	"	"	"	"	Asthma, cystitis and stone

# THE INFECTIVITY OF THE FLAGELLATE FORM OF *LEISHMANIA DONOVANI*

BY

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[Received for publication, June 8, 1927]

CHRISTOPHERS, SHORTT and BARRAUD (1926) carried out a number of infection experiments with leishmania cultures and white mice. They found that cultures of more than 6 days old when injected intraperitoneally always caused infection, but that younger cultures did not cause infection. In most instances in their series the mice received at least three injections at various intervals. They injected the contents on one NNN culture tube on each occasion irrespective of whether the tube contained scanty young forms or numerous old forms, so that there were two variables both of which had to be taken into consideration, a conclusion that the young forms of the flagellate were not infective was not justifiable.

I felt that it was worth while repeating these experiments on slightly different lines, giving single injections only, and the series of experiments reported below were undertaken. In the meanwhile, Shortt, Craighead, Khazan Chand and Swaminath (1926) were carrying out further experiments to clear up the ambiguity and when they reported their results, I felt that it was scarcely worth while continuing my series. The original intention had been to inoculate at least 100 mice, but only 66 had been inoculated up to that time. My results are perhaps worth recording, especially as the experiments were carried out on slightly different lines from those of other workers, but, as they are to a certain extent only complementary to those already reported by Shortt and his colleagues, they are reported as briefly as possible.

## DETAILS OF THE EXPERIMENTS

*The mice*—The mice were white, brown, or brown and white, bred from a stock imported from England, they were half to three-quarter grown at the time of inoculation.

*The cultures*—These were obtained from spleen puncture material from patients suffering from kala-azar. If very little material was obtained only one

protracted for a further 17 days in the case of the animals receiving milk in addition to the oatmeal diet, the survivors on this regimen (15) were killed by drowning on the 167th day. All animals were subjected to post-mortem examination.

#### RESULTS OF THE EXPERIMENT

The results of the experiment are given in the accompanying Table. They may be summarized as follows —

*The animals fed on the oatmeal diet without milk* grew badly, all died within periods ranging from 66 to 150 days. Nine (50 per cent) were found at post-mortem examination to have formed stones or gravel or both in the bladder, usually with, but in one case (686) without cystitis. Cystitis was present in one case (696) without associated stone or gravel. The sequelæ of calculus—dilated ureter, hydronephrosis and pyonephrosis—were not present in any case in this series, nor was stone found in the ureter or kidney. In one animal (699) a large grey-white, pear-shaped, fibinous mass was present in the bladder, the thin end being drawn out into a fine thread which extended into the urethra, this condition was associated with hæmaturia. Bloody urine was passed by two other animals (697, 698) in one of which cystitis was present in association with a number of minute stones (gravel). The three animals, in which hæmaturia was observed prior to death, were found at post-mortem examination to have suffered from a severe anæmia, associated with a lemon-yellow tinting of the skin and subcutaneous tissues. Three of the 18 rats in this group died of pneumonia, in three others no cause of death could be found except asthenia.

*The animals fed on the oatmeal diet with milk* grew well as a rule, though some better than others, this was no doubt due to the fact that some of them secured more of the milk ration. Two died of pneumonia, and one of an unknown cause. The other 15 remained in good health. Neither stone-in-the-bladder nor gravel nor cystitis nor any abnormality of the urinary tract was found at post-mortem examination in any of these 18 animals.

#### CONCLUSION

The addition of whole milk, in the proportion of two-thirds of an ounce per rat daily, to a diet capable of causing urolithiasis in 50 per cent of young rats completely prevented the development of phosphatic calculi.

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Serial number of mouse	Age of culture in days	Interval after which mouse was examined, in months	RESULT OF EXAMINATION	
			Smears	Cultures
7	10	4	Leishmania	Leishmania
8	10	4	"	Contaminated
20	18	4	"	Leishmania
21	11	3½	No parasites	"
22	5	4	Leishmania	"
25	11	4	"	"
27	10	4	"	"
30	22	3½	No parasites	"
34	9	3½	Leishmania	Contaminated
37	14	3	"	Leishmania
43	4	3½	No parasites	"
48	18	3	Leishmania	"
52	17	3½	No parasites	"
59	5	4	Leishmania	Contaminated
62	10	4	"	Leishmania

a comparison of the infection rates of the various groups as each group only contained such a small number of mice, these tentative conclusions are as follows —

That the early cultures in which the flagellates are scanty give rise to infection less readily than the older, richer cultures

That after 12 days or so there is little increase in the infectivity of a culture

That, as the parasites in the 12-day cultures are many times more numerous than those in the 5-day cultures and the infectivity of the former is only about twice that of the latter, the younger flagellate forms are actually more infective than the older ones

That there is a decrease in the infectivity of the individual flagellate after it has reached some age between 12 and 28 days

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in unison The fourth free flagellum arises from the same site but passes backwards and laterally, close to, but not attached to the undulating membrane, from which it is quite distinct

Wenyon (1926, p 669) has recorded the finding of a *Trichomonas* with three anterior flagella in the gut of an Indian python, but the present variety (or subspecies) with a fourth antero-lateral flagellum appears to be new

My thanks are due to Major R Knowles, I M S, Professor of Protozoology, Calcutta School of Tropical Medicine for much kindly help and advice

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### III

BY -

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[Received for publication, June 13, 1927]

In previous reports (1) (2) the experimental production of stone-in-the-bladder in rats was recorded. The composition of the calculus-producing diet was as follows —

Tinned Scotch oatmeal or <i>atta</i> (whole wheat flour)	53 parts
Linseed meal	20 "
Tinned cornflour	25 "
Sodium chloride	1 "
Calcium phosphate	1 "
Distilled water	<i>ad libitum</i>

The present report deals with the prevention of stone-in-the-bladder in rats fed on an 'oatmeal diet' of the above composition.

#### THE EXPERIMENT

Two groups of young rats, 18 in each, were selected, their aggregate body-weight was the same 910 grammes. There were 9 males and 9 females in each group. The body-weight of the males ranged from 50 to 58 grammes, of the females from 46 to 52 grammes, all were of approximately the same age. The 36 animals were confined in 12 precisely similar cages, there being 3 males or 3 females in each. All were fed on the oatmeal diet and water and were allowed to eat as much as they liked. One group received fresh whole milk in addition to the oatmeal diet, the other did not. A ration of 2 ounces of milk was provided for the three rats in each cage. Some secured more than their share of this ration. The average consumption of milk per rat daily was two-thirds of an ounce.

The experiment was continued until the death of all animals receiving the oatmeal diet without milk, the last two animals dying on the 150th day. It was



[illegible]

of which there are several on the market. The inorganic salts are slower in their effect and are not so effective, but they are at present infinitely cheaper and are therefore more commonly used. The organic salts are more effective but more expensive and some of them deteriorate on keeping.

We decided, therefore, to test samples from each group in the first instance. We chose, therefore, sodium antimony tartrate from the first group and (1) the most commonly used organic salt of antimony 'U' as well as (2) an organic compound 'X' which we had found effective in curing kala-azar though not as effective as 'U,' but which had proved non-irritant or only slightly irritant when injected under the skin of animals and a baby 14 months old suffering from kala-azar. This point was essential as we wished to use white mice in large numbers for the second experiment and they had to be injected every second day.

In the first experiment rabbits, each over 4 lbs in weight, were used and females only experimented on. We should have liked to have carried out all our experiments on rabbits as the drugs could be and were administered intravenously, but the impossibility of sparing a sufficient number of large breeding does at a Pasteur Institute in India made it impossible. We, therefore, had to select another animal. We were unable to use guinea-pigs as their long gestation period, three months, put them out of count. We, therefore, selected English white mice whose gestation period, three weeks, and their extremely numerous progeny made them a very suitable animal. The only trouble with these animals was the practical impossibility of injecting them every second day intravenously and the impossibility of mating them on known dates. We, therefore, gave them the organic salt of antimony 'X' hypodermically and judged the result on the actual number of young born. This result we divided into two divisions.

- (a) Young born during the first three weeks, i.e., in which the pregnancy had started before the antimony salt had been administered
- (b) Young born after three weeks, i.e., in which the pregnancy started after the treatment with antimony salt had commenced

With regard to this experiment, No 2, on white mice the following groups were made

- (1) 10 females and 2 males, only the females being injected
- (2) 10 females and 2 males, only the females being injected
- (3) 10 females and 2 males, only the males being injected
- (4) 10 females and 2 males, both females and males being injected
- (5) 10 females and 2 males, both females and males being injected

#### *Controls*

- (6) 10 females and 2 males, injected every other day with 1 c.c. normal saline

This experiment was carried out to exclude the factor of disturbance of the animal preventing pregnancy or causing abortions

- (7) 10 females and 2 males, not injected with anything
- (8) 10 females and 2 males, ditto but only observed for 30 days instead of 60 days
- (9) 10 females and no males, not injected with anything but just kept to prove that no births occur after three weeks if males are excluded

# NOTE ON A *TRICHOMONAS* FOUND IN THE GUT OF A NON-POISONOUS COLUBRINE SNAKE

BY

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[Received for publication, June 13, 1927]

IN the middle of last April a small colubrine snake was captured in the vicinity of the School, and was brought in for identification. It was identified by Lieutenant-Colonel H W Acton, I M S, as a young *Ablaber calamaria*. It was then chloroformed and the gut contents examined for protozoa.

The gut was practically empty in its whole extent, so some of the mucoid material was scrapped from the gut wall, emulsified in saline, examined under the microscope, and inoculated into Row's hæmoglobin-saline medium. The cultures were placed in the ice chest (temperature 16 to 20°C). No protozoa were seen in the direct smear.

Three days after inoculation the cultures were examined and showed a few trichomonads and very scanty Bodo. A few days later the cultures were found to contain a very large number of flagellates, the trichomonads predominating. Possibly the Bodo had been suppressed by the low temperature of incubation.

In addition to Row's medium, four other media were tried for subculture, viz, Lynch's serum-saline medium, Boeck and Drbohlav's medium, weak broth, and Locke's solution with egg albumin. In none of these media, however, was growth as satisfactory as in Row's medium. This fact I have also noted previously when cultivating *Trichomonas* of man. *Trichomonas hominis* appears to grow best at 37°C, but at this temperature bacterial growth is also heavy, and subcultures have to be made at intervals of two to three days to keep the *Trichomonas* strain going.

To study the morphology of the *Trichomonas* from the snake, smears were prepared and fixed in the wet state over osmic acid, and then stained by Shortt's technique with Giemsa's stain, as for *Leishmania* cultures. A careful examination of the smears, and also of the cultures under the dark ground, showed that the organism possessed four free flagella. The microphotograph was taken from a stained slide. A group of three intertwined flagella arise anteriorly and pulsate

No 2 Experiment carried out on white mice with Organic Antimony Compound 'X' injected hypodermically

Batch	Number of mice experimented on	Organic Antimony Compound 'X', 0.01 gm every alternate day	Total number of days experimented on	Number of young born during first 3 weeks	Number of young born after 3 weeks	Total number of young born	Number of adult mice which died during experimental period	REMARKS
No I Females only injected	10 females 2 males	39 injections	77	0	4	4	3 females not replaced	Observed for 40 days, after injections were discontinued 5 young born after treatment ceased Out of 16 young born, 15 died within 3 days of birth
No II Females only injected	10 females 2 males	30 injections	60	7	9	16	6 females died not replaced	
No III Males only injected	10 females 2 males	30 injections	60	24	13	37	1 female died not replaced	
No. IV Males and females injected	10 females 2 males	30 injections	60	9	11	20	6 females died, 1 male died, not replaced	16 young died within 3 days of birth
No V Males and females injected	10 females 2 males	30 injections	60	8	2	10	2 males died and replaced by 2 other males 1 female died and was not replaced.	
No VI Males and females injected	10 females 2 males	Normal saline solution 1 cc every other day 30 injections	60	27	59	86	1 female died immediately after being injected	Control (saline injections only)
No VII Control	10 females 2 males	Injections nil	60	25	29	54	Not replaced Nil	Control (no injection)
No VIII Control	10 females 2 males	Injections nil	30	10	7	17	Nil	Control 30 days only (No injections)
No IX Control	10 females No males	Injections nil	60	5	0	5	Nil	Control (no males)

PLATE XLVII







STUDIES OF THE EFFECTS OF ANTIMONY SALTS  
NO I THE EFFECT OF ANTIMONY SALTS ON CONCEPTION  
AND PREGNANCY IN ANIMALS

BY

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DURING the course of the treatment of a large number of cases of kala-azar at the Pasteur Institute and Medical Research Institute Hospital, Assam, by one of us with various salts of antimony, it struck us that there did not appear to be very much experimental work done of late years on the effect of antimony salts, given intravenously or hypodermically, on the various organs of the body itself. Considering the highly poisonous nature of these salts this seemed to us a mistake. A large number of experiments have undoubtedly been carried out to prove whether these drugs actually cause death to the animals they are injected into and what is the minimal lethal dose per body weight of the animal, but as regards their more remote effects on the body we were unable to find any recent references. Our determination to investigate this question was stimulated by the enquiries of some of the patients and their friends whether antimony salts caused sterility or abortion and by the obvious implication that they thought it had some such effect. The Director of Public Health, Assam, also informed one of us that during his tours of the Kala-azar Treatment Centres under his jurisdiction this suggestion had been made.

The importance of this question cannot be too strongly emphasised when figures show that over 60,000 patients were treated with antimony salts for kala-azar in Assam during the year 1925-26 and over 100,000 patients in Bengal.

The salts of antimony now used for the treatment of kala-azar on a large scale in India can be divided into two groups—the inorganic salts of antimony of which sodium antimony tartrate is used almost exclusively and the organic salts

well to enquire first whether there are any other diseases or conditions which have the same effect. It is found that almost any prolonged febrile condition, such as malaria, enteric, small-pox, staphylococcal or streptococcal infection, acts in a similar way. The injection of foreign material into the body such as turpentine, proteins, etc., if it causes febrile reaction, tends to be of benefit and in a degree largely corresponding to the height and the length of the febrile condition. This is only true, however, in cases in which there are very large numbers of lepra bacilli in the body, the reverse being the case if the numbers of bacilli are few.

Three explanations have been put forward as to the cause of the above —

(1) That the heightened temperature only prevents the growth of *B. lepræ*. This does not provide an adequate explanation, as many of the bacilli become very rapidly granular after the rise of temperature occurs, thus showing degeneration or death.

(2) Another suggestion is that the febrile condition acts directly on the bacilli causing their destruction. This, however, is negatived, as in early cases of leprosy when the bacilli are few in number, instead of being destroyed, they increase rapidly in numbers during the febrile condition or immediately after it subsides.

(3) It has been shown already that the improvement caused in leprosy by kala-azar is accompanied by the breaking down of *B. lepræ*. There are three other signs, however, which are present but have not been mentioned —

(a) The swelling up and reddening of existing lesions

(b) The formation of fresh lesions in the form of rose-coloured nodules

(c) Fever, which is superadded to the fever which has caused the reaction

The swelling of existing lesions shows that lepra bacilli, living or dead, or their products, have come in contact with the surrounding tissues. The formation of fresh nodular lesions in different parts of the body shows that lepra bacilli have been set free in the blood-stream and carried to end capillaries in the skin. The fever shows that certain bacillary products have been set free in the blood-stream and have caused a febrile reaction probably of an allergic nature. Whatever happens, therefore, when kala-azar and other febrile conditions cause the break-up and destruction of the leprous granuloma, it is evident that large numbers of bacilli, which had been isolated from the surrounding tissues, suddenly come in contact with these tissues, many of the bacilli reach the blood-stream and a certain amount of their products capable of causing fever is set free.

The nearest analogy is to be found in what takes place in the ague phase of benign tertian or quartan malaria. In malaria a parasite enters a cell, but is so non-toxic that it does not destroy by its secretions the cell it has entered, it multiplies inside that cell and then bursts, partly due to its own ripeness and, possibly, partly due to the explosion-producing power of the fever caused by the bursting of other similar cells. It then sets free fever-inducing products, and the freed parasites either settle down in new cells or are destroyed, according to the presence or absence of certain factors. In leprosy we have the lepra cell, which differs from the malaria-infested red cell in belonging to the solid and not to the liquid tissues. This cell comes to contain lepra bacilli which multiply inside it without secreting any substance which can destroy it. The cell is destroyed in the end as

## No 1 Experiment Intravenous Injection of (A) Sodium Antimony Tartrate (B) 'U' into Rabbits

Number of doe	Date mated	Date injections commenced	Drug and amount injected	Total number of injections	Result	Date again mated	Number of injections given	Result	REMARKS
1 A	20-10-26	12-11-26	Sod Ant. Tart 0.01 grm	7	Missed	28-11-26	0	4 young born, none destroyed	
2 A	20-10-26	12-11-26	do	10	Missed	20-12-26	0	Doe died on 17-1-27	
3 A	11-11-26	12-11-26	do	11	Doe aborted, 2 immature young born and doe died 8-12-26	27-12-26	12 Sod Ant. Tart	Missed	Injections continued over two matings
4 A	23-11-26	26-11-26	do	17	Missed				
1 B	22-10-26	11-11-26	Organic Ant 'U', 0.05 grm	9	Missed	28-11-26	0	3 young born, none destroyed	
2 B	25-10-26	11-11-26	do	7	Litter born but doe destroyed them all	29-11-26	0	8 young born, none destroyed	
3 B	11-11-26	11-11-26	do	16	Only placenta found	13-12-26	0	do	Not used a second time.
4 B	23-11-26	26-11-26	do	15	5 young born, 2 destroyed				

## CONTROLS

1 C	20-10-26		Nil		4 young born
2 C	28-11-26		Nil		Missed
3 C	11-12-26		Nil		3 young born
4 C	12-12-26		Nil		Missed



Number (1) group was further observed for 40 days after cessation of the organic antimony compound 'X' injections. During this period the seven remaining females gave birth to five young.

As regards the first experiment on rabbits this was divided into two divisions

(A) those rabbits injected intravenously with sodium antimony tartrate,

(B) those rabbits injected intravenously with the organic antimony compound 'U'

The results of these experiments are set out in the two tables attached

We recognise that the number of experiments is small and that the results are not therefore absolutely decisive and the time at our disposal, three months, was not sufficient, but we considered our results were sufficiently striking to put forward so that other workers interested in the same problem might be encouraged to further investigate this subject

Our conclusions from these experiments were

(1) there is definite evidence to show that salts of antimony do cause damage to female animals while they are receiving these periodic injections. That this damage shows itself in either

(a) failure of conception,

(b) abortion,

(c) damage to the foetus,

generally in all three ways

(2) This injury appears to pass off very rapidly after the injections of antimony are stopped

(3) There is not sufficient evidence in our experiments to show that antimony salts have any effect in causing sterility in males

section is shown in the photomicrograph (Plate XLVIII, fig 1) The intima has proliferated and caused considerable swelling which increases the size of the vein and by bulging into the lumen causes obliteration At times the vein may be obliterated at various points while the lumen between is filled with blood, either clotted or grumous Later the vein is reduced to a thin, fibrous chord and then disappears When sodium hydnocarpate is given subcutaneously much pain is caused

Many attempts have been made to overcome this difficulty and lately under the instructions of Sir Leonard Rogers a sodium salt has been prepared from the low melting-point fatty acids of hydnocarpus oil which is very much less irritating and can be given in a one per cent solution either subcutaneously or intravenously As Sir Leonard Rogers points out small doses often produce the required reaction and in many cases the small dose necessary to cause such a reaction, and therefore the maximum advisable dose, can be continued for a long time without blocking of veins when given intravenously or without pain when injected subcutaneously

It is, however, often advisable to give large doses and to use a solution stronger than one per cent as in the following circumstances —

(a) In early cases in which there are only one or two anæsthetic patches In such cases much more rapid improvement may be hoped for with large doses

(b) In any cases in which careful treatment has caused improvement of the general health, in which the muscles are firm and well-developed and the general resistance high Reactions are difficult to produce in such cases but more rapid improvement is produced by large doses

(c) In cases in which there are very large numbers of bacilli in the skin In such cases large doses will produce marked reactions and the breaking down of leprous tissue which appears to induce a high degree of temporary immunity and in many cases causes the rapid destruction of bacilli and marked improvement of the condition of the patient, and that even though the general health of the patient may not be very good at the time

If it is conceded that a water-soluble preparation like sodium hydnocarpate is the ideal preparation for the administration of hydnocarpus oil, it is very important that a method of giving large doses of it painlessly should be discovered

We believe that we have to a large extent succeeded in doing this by a very simple device A 20 c.c. syringe (with central nozzle) is used with a sharp needle of fairly large bore A 2 per cent solution of sodium hydnocarpate prepared from the whole oil is used The dose to be administered, 2 to 8 c.c., is placed in the syringe after sterilising it by sucking up and evacuating oil at a temperature of 130° centigrade 3 or 4 times A rubber band is passed round the arm which is held out at right angles to the body

A prominent vein is pierced by the needle and blood varying from an equal quantity to double the amount of the solution to be administered, is sucked up into

# THE EFFECT OF KALA-AZAR ON LEPROSY.

BY

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So many remedies for leprosy are put forward nowadays that it may be useful to mention our experience of the effect of kala-azar in leprosy. For, although kala-azar is a debilitating disease, yet in the third stage of leprosy (B3 cases) in which the skin is loaded with lepra cells full of bacilli it produces a very marked and rapid improvement in the leprosy condition.

Kala-azar has evidently been endemic in the Albert Victor Leper Hospital, Gobra, Calcutta, for some considerable time. During the six years that I have been associated with it we have diagnosed no less than 16 cases among the inmates. In five of these cases the diagnosis was made by finding the parasites in spleen puncture smears and in 14 cases the 'aldehyde test' was found strongly positive. One typical case was only diagnosed clinically. Of the 16 cases 3 died, one of them of tuberculosis and two of septicaemia who had previous to the kala-azar been in a very bad condition. The other 13 cases recovered from kala-azar under treatment and in every one of them there was a marked improvement in their leprosy. Leprous lesions either entirely disappeared or were very much reduced in size, lepra bacilli became granular. In short the patients invariably improved both clinically and histologically. So striking was the improvement that volunteers from among the leper patients came forward offering themselves for inoculation with kala-azar.

In contrast to the above cases must be mentioned 5 early cases in which leprosy appeared during the latter part of treatment for kala-azar. As long as the fever lasted there were no signs of the former disease, but once the fever had disappeared signs of leprosy began to show themselves. In three of these cases there were marks showing that slight leprosy lesions had been present before and had been lit up by the lowered general resistance resulting from the kala-azar.

*Discussion*—In seeking to interpret the phenomenon of the breaking up and destruction of leprosy granulomatous tissue under the influence of kala-azar it is





in malaria by mere mechanical pressure from within. It reaches the bursting point and in the ordinary course a few lepra cells burst from time to time without causing any noticeable reaction. But a rise of temperature has the effect of causing the simultaneous bursting of large numbers of lepra cells, and their bacillary contents are brought into contact with the surrounding tissues, some bacilli are set free in the blood-stream and a febrile reaction is caused, which in turn may cause still further bursting of other lepra cells, which, meantime, have reached maturity or required greater or more prolonged fever to cause their rupture.

If we accept the above theory as to what happens when some factor such as the fever of kala-azar causes a leprosy reaction, we have still to explain why in the earlier stage of leprosy this febrile disease should be followed by an increase of the symptoms of leprosy, while in the third stage, when leprosy is at its zenith, it is accompanied by rapid elimination of that infection from the body. Here probably the analogy between leprosy and malaria ceases, for we are dealing not with a protozoal disease like the latter, but with a bacterial disease, which is capable of inducing immunity, though it may be tardily.

What happens then when a reaction is produced in the third stage of leprosy is that very large numbers of bacilli are brought in contact with the tissues of the body by the bursting of lepra cells, and a high degree of special immunity is induced thereby, with the result that bacilli are destroyed by phagocytes which invade the granuloma, and bacilli which are carried to other parts of the body in the blood stream are also destroyed. It is noticed that in such cases the fresh, rose-coloured nodules which appear, disappear again in a few days. In the first and second stages, however, when bacilli are comparatively few in number, although a certain number of them are brought in contact with the tissues of the body, a sufficient amount of immunity is not induced to cause destruction of bacilli, hence the *B. lepra*, when freed from cells, form fresh colonies and the disease extends. This is helped still further by the lowered resistance which is caused by kala-azar or other diseases.

The immunity of leprosy appears to be caused tardily by the setting free of very large numbers of *B. lepra*, and it tends to die down again to a certain extent if the supply of freed bacilli is not kept up, although a smaller amount of immunity persists for a very long time.

Although the above theory is set forth as being apparently the only possible one, it is admitted that it is difficult to prove immunity in leprosy directly by serological methods so long as it is not possible to culture the causal organism *in vitro*.

We may state, therefore, that the effect of kala-azar in leprosy is to cause auto-vaccination of the patient, a process which may be dangerous or harmful in the earlier stages, but is decidedly beneficial as far as the treatment of leprosy is concerned in the third stage of typical skin cases.

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gives an idea of the relative hæmolytic power of these oils diluted in different strengths with normal saline —

Dilution		1—3000	1—5000	1—6000	1—7000	1—8000
Sodium salt of oil of	<i>H wightiana</i>	++	+	+	—	—
„	<i>H wightiana</i> (B & W)	++	+	+	+	—
„	<i>H alpina</i>	+	—	—	—	—
„	<i>H anthelmintica</i>	+	—	—	—	—
„	linseed	+	+	+	+	+
„	olive	++	+	+	+	+
++		= immediate hæmolysis				
+		= delayed hæmolysis				

This experiment suggested that, seeing the hæmolytic power of sodium hydnocarpate *alpina* and *anthelmintica* was considerably less than that of other soap solutions, these salts might have a lesser vein-blocking power than the corresponding salts of *H wightiana*. This was found to be the case with sodium hydnocarpus *alpina*, as a 2 per cent solution was administered, without mixing with blood, in doses rising from 4 to 10 c c, 10 injections being given into one point of the same vein before blocking occurred. *H anthelmintica* was also found to have considerably less vein-blocking power *H wightiana* though not as little as *H alpina*.

While the sodium salts of *H alpina* and *H anthelmintica* are, therefore, preferable to those of *H wightiana* when the solution is given directly into the vein without mixing with blood, the latter salt can be given with equal facility and for as long a period as is necessary when mixed with blood as described above.

Our usual routine at present in Calcutta is to give injections of pure *H wightiana* oil in gradually increasing doses up to 10 c c by the infiltration method and then to give sodium hydnocarpate intravenously in a 2 per cent solution by the blood-mixing method. Given in this way the drug may be continued for a considerable period. We seldom find it expedient to go beyond 8 c c of a 2 per cent solution as higher doses are apt to cause a feeling of giddiness. Because of the hæmolytic power of sodium hydnocarpate solution, however, it is well to substitute the subcutaneous infiltration of the oil with creosote again from time to time.

### SUMMARY

1 While the intravenous injection of sodium hydnocarpate may be admitted as the most effective means of administering hydnocarpus preparations, the blocking of veins has hitherto interfered with prolonged administration in this way.

2 The sodium salts derived from a special fraction of *H wightiana* and also the sodium salts of *H anthelmintica* and *alpina* block the veins less than the salts from the whole *H wightiana* oil.

3 A new method of mixing the salts prepared from the whole oil of *H wightiana* with blood before injection has reduced vein-blocking to a much more marked extent.

# SODIUM HYDNOCARPATE IN LEPROSY, SUGGESTED IMPROVEMENTS IN ADMINISTRATION

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HYDNOCARPUS (Chaulmoogra) oil has been used for many centuries in India in the treatment of leprosy. The original methods of administration were oral and byunction. In 1913 Heisser reported good results with intramuscular injection of the oil (Mercardo's formula). But a very distinct advance was made when Sir Leonard Rogers introduced the intravenous injection of sodium hydnocarpate in 1915. It stands to reason that a water-soluble preparation should furnish the most effective method of administration. The difficulty, however, was that sodium hydnocarpate very quickly caused blocking of the veins so that the treatment had to be suspended for want of superficial veins to inject it into.

The cause of this vein-blocking was an endophlebitis of the injected veins, often 5 or 6 inches of the vessel beyond the site of injection becoming impervious. The changes which take place in a vein when injected with sodium hydnocarpate are immediate and delayed —

(a) The immediate signs are not always present, but, when they are, there is pain along the vein proximal to the point of injection for about 3 to 6 inches and the vessel stands out as a hard cord. Both of these effects pass off more or less after 10 or 20 minutes though a certain amount of hardness and a dull aching pain may persist. In other cases there is no immediate hardening of the nerve but considerable pain comes on and may last for several hours.

(b) The delayed effects show themselves clinically as a thickening and hardening of the vein and obstruction of its lumen. The appearance on



PLATE XLVIII

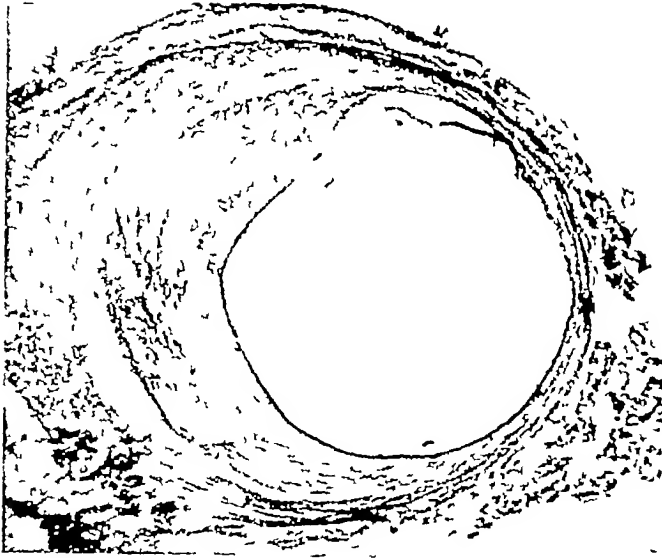


FIG 1—Section of vein wall thickened due to injection of sodium hydnocarpate solution

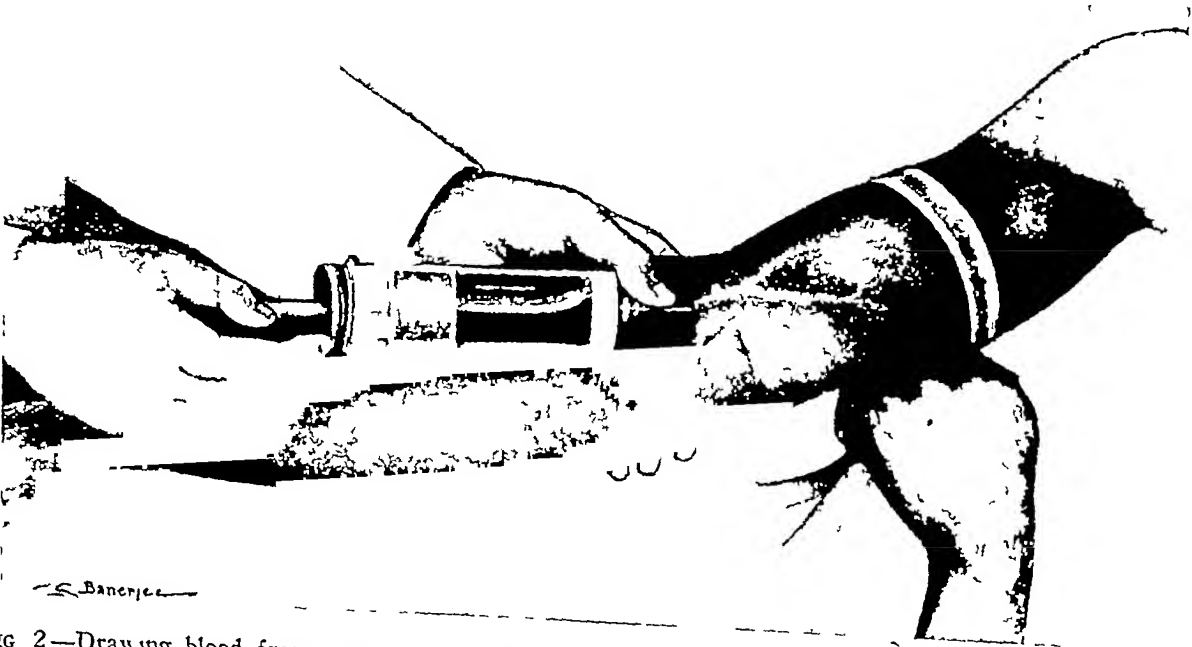


FIG 2—Drawing blood from vein to mix with sodium hydnocarpate solution The solution rises above the blood as it is lighter

- 3 Fever, not always present
- 4 Marked acceleration of blood sedimentation
- 5 Apparent granulation of lepra bacilli in lesions

If rose-coloured nodules appear and disappear again in a few days the physician can press the treatment with some assurance, as the disappearance is a sign of immunity and when this is present the breaking up of granulo-matous tissue and setting free of bacilli in the general circulation will not cause further dissemination of active disease but a gradual healing up of lesions

This treatment is suitable for all the stages and types of leprosy. In early cases the first sign of reaction produced by small doses may be the acceleration of blood sedimentation. In many, however, larger doses produce other signs such as the swelling up of affected nerves and induction of pain and tenderness in them.

In the second and third stages in which lepromatous tissue is abundant even the smallest doses produce all the five signs of reaction, though occasionally fever is absent entirely or during some of the reactions. In such cases, as the leprous tissue clears up under potassium iodide, the sedimentation gradually becomes retarded and the general condition of the patient improves. The patient has by this time reached a condition in which a comparatively large dose of potassium iodide (20 to 30 grains) is required to produce a reaction. At this stage it is well to give the drug once or twice a week in a dose sufficient to cause a moderate crop of nodules and a temperature rise of 2 to 4 degrees lasting for 24 to 48 hours. Such a reaction, when induced only once or twice a week, is generally tolerated by the patient. The treatment is continued until very large doses, 180 to 240 grains, can be administered without causing reaction and this massive dose is continued for some months.

As severe and prolonged reactions may be caused by potassium iodide it is important that we should have means of controlling such. The salts of several of the heavy metals have this effect. We have found that potassium antimony tartrate, given in small doses of 0.02 grām intravenously every second day, will control reactions and that adrenaline, 3 minims of the 1-1,000 solution (P. D. & Co.) in 30 minims of saline given subcutaneously or intramuscularly, causes immediate cessation of nerve pains.

#### POSLOGY

1 Begin with 1 grain of potassium iodide a day and increase the dose daily by one grain until there is a rise of temperature to more than 99° or till there is swelling and redness of the lesions in the skin. This is a safe rule, but with experience it is possible to begin with a larger dose in early cases and to increase it more rapidly.

2 When the temperature has become less than 99° and the swelling has begun to diminish, continue the iodide giving the same dose as produced the fever and swelling. Only give it now once or twice a week and not daily.

the syringe, which is held horizontally (Plate XLVIII, fig 2) The specific gravity of the sodium hydnocarpate solution being less than that of blood, the former rises to the top and the latter falls to the bottom The blood may either be allowed to force itself into the syringe by its own pressure or the piston may be gently pulled out by hand When the right quantity of blood has entered the syringe and without withdrawing the needle from the vein, the syringe, which is still held horizontal, is rotated in its long axis through  $180^\circ$  The blood and solution which adhere to a certain extent to the barrel of the syringe reverse their relative positions and the solution passes for a second under the blood It very soon, however, tends to rise to the top and in doing so mixes with the blood After a few seconds the syringe is rotated back again in the opposite direction to its original position If necessary this process may be repeated In this way the mixing is rendered complete While the mixing has been going on blood has still been slowly entering the syringe and one is thereby assured that the point of the needle is still in the lumen of the vein The band is then removed from the arm and the piston is gradually pressed home The solution has the effect of preventing the coagulation of the blood and though we have administered the drug in this way thousands of times we have never yet noticed clotting, nor has any untoward symptom occurred which might be due to injection of clots We have given 3 per cent sodium hydnocarpate solution in this way 10 or 12 times and large doses of a 2 per cent solution over 30 times into the same point in the same vein without any blocking of the vein occurring

To test the irritating power of sodium hydnocarpate prepared from the whole fatty acids and given in this way with that of the special fraction prepared by Messrs Burroughs Wellcome & Co referred to above, the following experiment was carried out A vein was chosen at the bend of the elbow and repeated injections of a 1 per cent solution of the fractional salt were given in the usual manner without mixing it with blood At the time a similar quantity of a 2 per cent solution of the salt, prepared from the fatty acids of the whole oil was given into the corresponding vein of the other arm, the solution being mixed with blood as described above It was found that whereas injections of the weaker solution of the less irritating salt blocked the vein, in not one case were the veins blocked by the blood-mixed solution It was also found that if instead of injecting the blood-sodium hydnocarpate mixture into the vein it was injected subcutaneously, no irritation was caused although the same solution without blood or serum was very irritating

Another possibility which occurred to us was that though vein-blocking is caused by the sodium salts of *Hydnocarpus wightiana* oil the salts prepared from the oils of other hydnocarpus species might not have this property to such an extent This possibility was also suggested by the fact that the salts of the oils derived from two other species of *Hydnocarpus* (*H. anthelmintica* and *H. alpina*) have less hæmolytic power than those derived from *H. wightiana* oil Sodium hydnocarpate like the sodium salts of other oils is highly hæmolytic This is not due to their being alkaline as no hæmolysis occurred in solutions of NaOH, which were considerably more alkaline than the soap solutions The following table



other signs of reaction Doses increasing from 30 to 240 grains should be given to adults and proportionate doses to children

Another important diagnostic use is in cases which have been under treatment with other remedies and in which all apparent signs have disappeared A patient should never be discharged as relatively or possibly cured until he has stood the test of massive doses of potassium iodide without showing any signs of reaction, as it has the power of searching out the disease wherever it may be in the body and showing it up

So far there has been no drug available in leprosy with any prophylactic power There is reason to believe that the occasional administration of iodides, in moderate doses working up to massive ones, to contacts and to those who have to work in leprosy institutions may be found useful in this respect

It is difficult to say in what way potassium iodide causes breaking down of leprous tissue with consequent reactions The action is apparently not a direct attack on the lepra bacillus nor is it to any extent a stimulation of cells of the body to attack the causal organism What probably happens is that in some way or other potassium iodide has the effect of removing the protective mechanism which shuts off the bacilli from the tissues

4 Alternation of intravenous sodium hydnocarpate with subcutaneous infiltration of hydnocarpus oil with creosote is advised-chiefly because of the hæmolytic effect of the former preparation

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the pharynx arise from the head capsule. Very often the three anterior dark spots just behind the bases of the frontal hairs are enveloped in a cloud forming a broad band of dark pigment in the middle of the head.

*Clypeal Hairs* (Plate XLIX, fig 1) the *inner anterior clypeals* (I A C) are about 222  $\mu$ m long, separated at their base by a distance equal to about one-third of the anterior width of the head. They bear a large number of fine hairs with a pinnate distribution\* along the distal three-fourth of their length. The *outer anterior clypeals* (O A C) are about two-thirds the length of the inner anteriors. They are branched in the distal half, bearing on each side from 5 to 8 branches which arise regularly from the shaft. The proximal branches are longer than the distal ones. The *posterior clypeal hairs* (P C) are about half the length of the outer anterior hairs and divide near the base into 2 to 4 branches which often reach beyond the bases of the inner anterior hairs. The six *frontal hairs* are long, bearing on each side 8 to 12 rather long branches. The *inner occipital hair* is about as long as the outer anterior clypeal and is simple, while the outer is branched from its base into 3 to 5 branches.

*Mentum* (Plate XLIX, fig 4) bears the usual large terminal tooth with a row of three on each side. The two anterior teeth of the row are placed just behind the terminal tooth while the third lies further back. *Submentum* (Plate XLIX, fig 6) the terminal tooth is double with a row of 4 to 6 teeth on each side.

*Thorax* the inner two *Submedian hairs* (Plate XLIX, fig 8) have thickened stems, that of the innermost being much dilated in the proximal half. They arise from a large strongly chitinated tubercle apparently formed by the union of their roots. Both are branched, the inner having 8 to 12 close-set branches on each side while the intermediate has 8 to 9 branches internally and 4 to 6 externally. The third or outermost hair is simple and arises by the side of the tubercle. The *palmate hair* (Plate L, fig 10) is well developed† with 6 to 11 long pointed leaflets, the filament is not differentiated.

*Abdomen* the *palmate hair* of the first abdominal segment (Plate L, fig 12) is very poorly developed having 5 to 9 long narrow leaflets. In most of the specimens it has the appearance of an ordinary branching hair. Segments 2 to 7 bear well-developed palmate hairs, which have 11 to 14 leaflets on the second segment (Plate L, fig 15) but 15 to 21 on the others. The average length of the leaflets on the mid-abdominal segments is 0.575 mm and the length of the filament about 0.222 mm. The filaments are pointed, those of segments 3 to 7 deeply indented at their base. *Dorsal plaques* are of moderate size. The *spiracular comb* (Plate L, fig 18, Sp C) is strongly chitinated and has 3 to 5 long and 10 to 12 short pointed processes all of which are serrated near their bases. The *post-spiracular hair* (P sp H) is well-developed having six or seven branches, four of which are very long.

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\* Unless otherwise stated all the branching of hairs described in this paper is *pinnate* in arrangement and not *plumose*.

† A 'well-developed' thoracic palmate hair is more or less like a poorly developed palmate hair of the abdomen and never like a 'well-developed' palmate hair of a mid-abdominal segment.

# THE IODIDE-ANTIMONY TREATMENT OF LEPROSY

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It has long been known that the iodides, especially the potassium salt, have a special action in leprosy. Most workers have regarded the apparent exacerbation produced by this drug as dangerous and a thing to be avoided. A few workers have considered it useful, especially in diagnosis, because they found that the nasal catarrh produced facilitated the search for lepra bacilli in the nasal secretion.

The present writer considers that the reaction produced by potassium iodide is not necessarily harmful, and that the breaking down of leprous tissue caused by the administration of the drug, if the dosage is wisely regulated, may be one of the most beneficial processes possible in the treatment of the disease.

Potassium iodide does not lend itself to use in mass treatment. It is necessary to begin with small doses, gradually increased according to the tolerance of the patient. Less than one grain may cause a febrile reaction which will last 2 or 3 weeks, while later on as the condition improves such massive doses as 240 grains a day may be taken without reaction.

In all stages of leprosy potassium iodide is a most useful therapeutic agent but in cases in which a considerable amount of leprous granulomatous tissue has been formed the breaking down of this tissue by potassium iodide apparently induces a considerable degree of immunity and these two factors (the breaking down of leprous tissue and immunity) combined are in our experience more powerful therapeutic agents in leprosy than any others which we have seen so far.

The reaction signs are the following —

- 1 Swelling up and erythema of existing lesions
- 2 The appearance of fresh, rose-coloured nodules which are often painful

*Mentum* unlike the mentum of the larva of *A. fuliginosus* which has seven teeth (Plate XLIX fig 4) that of this species has nine teeth (one median and a row of four on each side) Three teeth on each side of the median tooth are placed close together while the fourth is placed further back

*Thorax* tubercles of the inner two submedian hairs are not strongly chitinised as they are in *fuliginosus* and are often separate from each other The palmate hair is well-developed bearing 11 to 15 lanceolate leaflets The filament is not developed

*Abdomen* palmate hairs on the first segment bear 8 to 13 narrow lanceolate leaflets with poorly developed filaments, the indentations, marking the beginning of the filaments, being very small Segments 2 to 7 bear well-developed palmate hairs, having 15 to 25 broad leaflets with long, narrow, pointed filaments The average length of the leaflet of a mid-abdominal palmate hair is 0.519 mm and that of the filament is 0.411 mm

The *spiracular comb* has 4 to 5 long and 12 to 15 short spinous projections all of which are serrated at their bases The *post-spiracular* hair is well developed

#### LARVA OF *A. philippinensis* LUDLOW 1901 (?)

Out of a large number of adults, of *A. pallidus* Theobald, reared by me at Yellapur (North Kanara, Bombay Presidency) in March this year, two mosquitoes (one male and one female) differ from the rest in certain characters, which remind one of *A. philippinensis* Ludlow\* These as well as certain other important characters are described below —

(1) Wing The subcostal pale area is not bridged with dark on the first longitudinal vein

(2) Wing The 5th vein is pale all along its length except for a small dark area at its base and two on its anterior and one at the end of the posterior branch

(3) Prothoracic lobes without any pale scales, dark hairs only being present

(4) Mesothoracic pleuræ with a few pale scales, not very conspicuous

(5) Distal end of the first hind tarsal segment picked out with white (but not so prominent as in *fuliginosus*)

(6) In the female specimen three and a quarter while in the male three and one-third distal hind tarsi completely white

(7) A few pale scales present on the dorsum of the 6th abdominal segment, the 7th covered with pale golden scales

(8) Ventral surface of the abdomen without any scattered pale scales, dark hairs only being present Tuft of black scales on the venter of the 7th segment, inconspicuous

Characters 1 and 2 separate these two specimens from *A. fuliginosus* Giles while 3, 5 and 8 separate them from *A. pallidus* Theobald, these being according to Christophers (1924), the diagnostic characters of *A. philippinensis* Ludlow

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\* Among a large number of anopheline mosquitoes caught and reared by Lieut-Col T C McCombie Young, I M S, while making a malarial survey of Coorg, Western Ghats, in June and July last, there are 12 adults (5 males and 7 females) which resemble those two from Yellapur

3 When there is no fever or swelling after administration increase the next dose by 1 grain When there is fever or swelling do not increase the dose

4 If the fever or a great amount of swelling lasts for more than three days give potassium antimony tartrate 0.02 gram in 2 c.c. of sterile saline intravenously every second day till the fever comes down or swelling becomes less, then stop the antimony and begin the potassium iodide again

5 When the dose of iodide has reached 20 grains increase it by 5 grains instead of by one grain, the same indications for increasing the dose being followed as before

6 When 60 grains has been reached the dose may be increased by 30 grains in the same way, gradually rising to 120 grains When this amount causes no reaction 240 grains should be given and continued twice a week for three periods of one month each with a rest of one month without iodide after each period

7 Potassium iodide should be given dissolved in one or more large glasses of water at bed time When the dose is more than 60 grains it can be divided into two, half being taken after the midday meal and half at bed time

8 When the fever and marked swelling last more than 48 hours, iodide should be given only once a week, when they last less than 48 hours then twice a week

9 If the patient feels very weak, a rest from iodide may be given occasionally, but it is important to carry on the treatment without intermission from beginning to end as far as possible

10 Potassium iodide may be prescribed in the form of a mixture but it is more convenient to prescribe it in the form of 1, 5 or 30 grain tablets according to the size of the dose which is being taken

11 Between 5 grains and 30 grains there are sometimes symptoms of iodism or there is even an iodide rash, but these do not occur as a rule when the dose is larger than 40 grains, and in our experience they give very little trouble if the drug is taken as advised above

12 If there is a painful nerve reaction 3 minims of a 1-1,000 solution of adrenaline chlor (P. D. & Co.) in 30 minims of saline should be given intramuscularly and the dose repeated in 5 minutes if the nerve pains have not subsided in that time

13 The patient's temperature must be regularly taken and recorded at least 4 times a day, otherwise it is difficult to regulate the dosage

14 Generally speaking, the lowest reaction-causing dose and the rate at which the amount of the drug can be increased vary in inverse ratio to the number of bacilli in the body

An equally important use of potassium iodide is in making a diagnosis in early cases where there are suspicious signs and in testing contacts with infectious cases even when they show no such signs The sedimentation should be tested before the administration of the drug and again 24 hours after The temperature should be taken regularly and a careful search made for

fig 7) ends in a single median tooth instead of the double tooth found in both the latter species

*Thorax* the innermost submedian hair (Plate XLIX, fig 9) is short with 8 to 10 branches and has no conspicuous root The intermediate hair has a small dark root, and bears 6 to 12 branches on each side, the outermost hair is simple The palmate hair (Plate L, fig 11) is vestigial, having the form of an ordinary hair with 2 to 4 long branches

*Abdomen* palmate hair (Plate L, fig 14) on the first segment is vestigial, that on the second segment (Plate L, fig 17) is poorly developed with 6 to 9 lanceolate leaflets devoid of filaments On segments 3 to 7 the palmate hairs are well-developed bearing from 11 to 21 leaflets with short pointed filaments The average length of a leaflet from a mid-abdominal segment is 0.371 mm and that of the filament is 0.111 mm The spiracular comb (Sp C, Plate L, fig 19) has three long and eight small spinous projections, nearly all of which are serrated at their bases The post-spiracular hair has only four branches, two short and two very long

*Anopheles ramsayi* is very restricted in its distribution, being recorded so far only from Eastern Bengal, Assam and Burma where *A jamesi* is rare Although the adults of the two species are quite distinct, the former was not recognised as being a different species from the latter till quite recently

Covell (April, 1927) in his description of the adult of this species has shown that it is markedly different from *A jamesi* in a number of characters, which have been tabulated below Strickland and Chaudhury (May, 1927) have described, from Bengal the larva of a new species which they have called *A pseudojamesi* Regarding the adult of this latter species these authors describe slight differences in the amount of white scaling on the hind legs and state that in all respects, except in the male genitalia the species is similar to *A jamesi* Their figure of male genitalia of *A pseudojamesi* is identical with the description given by Covell for *A ramsayi* Although Strickland and Chaudhury differ from Covell in saying that except for the genitalia and the hind legs the adults of *A pseudojamesi* and *A jamesi* are alike, yet it appears that they have described the same species as Covell, since a close study of the larva of *A ramsayi* has shown that this is identical with the larval characters of *A pseudojamesi* as described by Strickland and Chaudhury

The chief points of difference between the adults of *A jamesi* and *A ramsayi* are —

<i>A jamesi</i>	<i>A ramsayi</i>
(1) A medium sized mosquito, length of wing about 3.5 mm	A small mosquito, length of wing about 2.65 mm
(2) Wing markings tend to resemble those of <i>A stephensi</i> Both the inner quarter and the outer third of costa chiefly pale wing field mainly pale, the dark spots being very small in extent	Wing markings tend to resemble those of <i>A fuliginosus</i> Both the inner quarter and the outer third of costa chiefly black wing field mainly dark

A NOTE ON THE FULL-GROWN LARVÆ OF *ANOPHELES*  
*JAMESII* THEOBALD, *A. FULIGINOSUS* GILES,  
*A. PALLIDUS* THEOBALD AND *A. RAMSAYI*  
COVELL (CULICIDÆ, DIPTERA)

BY

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SOME interest attaches to the validity or otherwise of the closely related species, *A. fuliginosus* Giles (and its variety *adiei*), *A. pallidus* Theobald and *A. philippinensis* Ludlow, and also of *A. jamesi* Theobald and the recently described *A. ramsayi* Covell. The following is a brief note on the larval characters of these forms (except *A. philippinensis* of which larval material has not been available to me so far).

All these forms (except *adiei*) are recognised as distinct species by Christophers (1924), but Dyar and Shannon (1925), doubting the presence or absence of pale scales on the venter of the abdomen of adult mosquitoes to be a constant character, regard *philippinensis* as synonymous with *pallidus*.

A close study of the larvæ of all the Indian species of Anophelines is at present being made by me, but in the meantime, observations on the larvæ of the abovenamed four species will be of interest. The following descriptions have been based on the moults of the final larval instars in all cases. Except as regards *A. philippinensis* (?) and *A. ramsayi* where 2 and 3 larvæ only were respectively available, 25 larvæ of verified adults of each species have been carefully compared in detail.

LARVA OF *Anopheles jamesi* THEOBALD 1901

The head is golden brown in colour with the usual dark brown spots (sometimes very dark), indicating the places where the muscles of the mouth-parts and



# EXPLANATION OF PLATE XLIX

- Fig 1 Anterior portion of the Clypeus of *A jamesi* showing the clypeal hairs  
Scale A
- „ 2 Anterior portion of the Clypeus, *A fuliginosus* showing the clypeal  
hairs Scale A (Simple posterior clypeal hair is exceptional )
- „ 3 Anterior portion of the Clypeus, *A ramsayi* showing the clypeal hairs
- „ 4 *Mentum* of *A jamesi* Scale B
- „ 5 „ of *A ramsayi* „
- „ 6 *Submentum* of *A jamesi* „
- „ 7 „ of *A ramsayi* „
- „ 8 Submedian Thoracic hairs of the right side *A jamesi* Scale A
- „ 9 Submedian Thoracic hairs of the right side *A ramsayi* Scale A

LARVA OF *A fuliginosus* GILES 1901

The larva of this species resembles very closely that of *A jamesu*, from which it can be differentiated with great difficulty. It differs from the latter only in minor details, which are herein described.

**Head** (Plate XLIX, fig 2) the branches of the clypeal hairs do not arise as regularly along the shaft of the hair as they do in *A jamesu* and tend to cluster towards the distal end of the hair. The inner occipital hair is simple in this species also, although occasionally it is bifid near its distal end. This hair has been described as simple by Swellengrebel (1919), while according to Carter (1925) and Stanton (1915, 1927) it is branched 4 to 5 times. Strickland (1925) recorded it to be branched, but Chaudhury (Strickland and Chaudhury, 1927) found it to be simple in a majority of cases. The figure of *A fuliginosus* Giles given by Stanton (page 73, 1927) is undoubtedly that of *A philippinensis* Ludlow and it is highly probable that he has described the larva of that species under *A fuliginosus* Giles. Whether the others were dealing with the larvæ of *A fuliginosus* or of *A philippinensis* or even of *A pallidus*, it is very difficult to say (the figure of the adult given by Carter is, however, that of *fuliginosus*).

The branches of all the hairs of the head are comparatively longer than those in *jamesu*.

**Thorax** in a considerable number of specimens, the innermost submedian thoracic hair is comparatively longer, but less swollen. The palmate hair is well-developed bearing from 2 to 11 broad, pointed lanceolate leaflets, filaments undifferentiated.

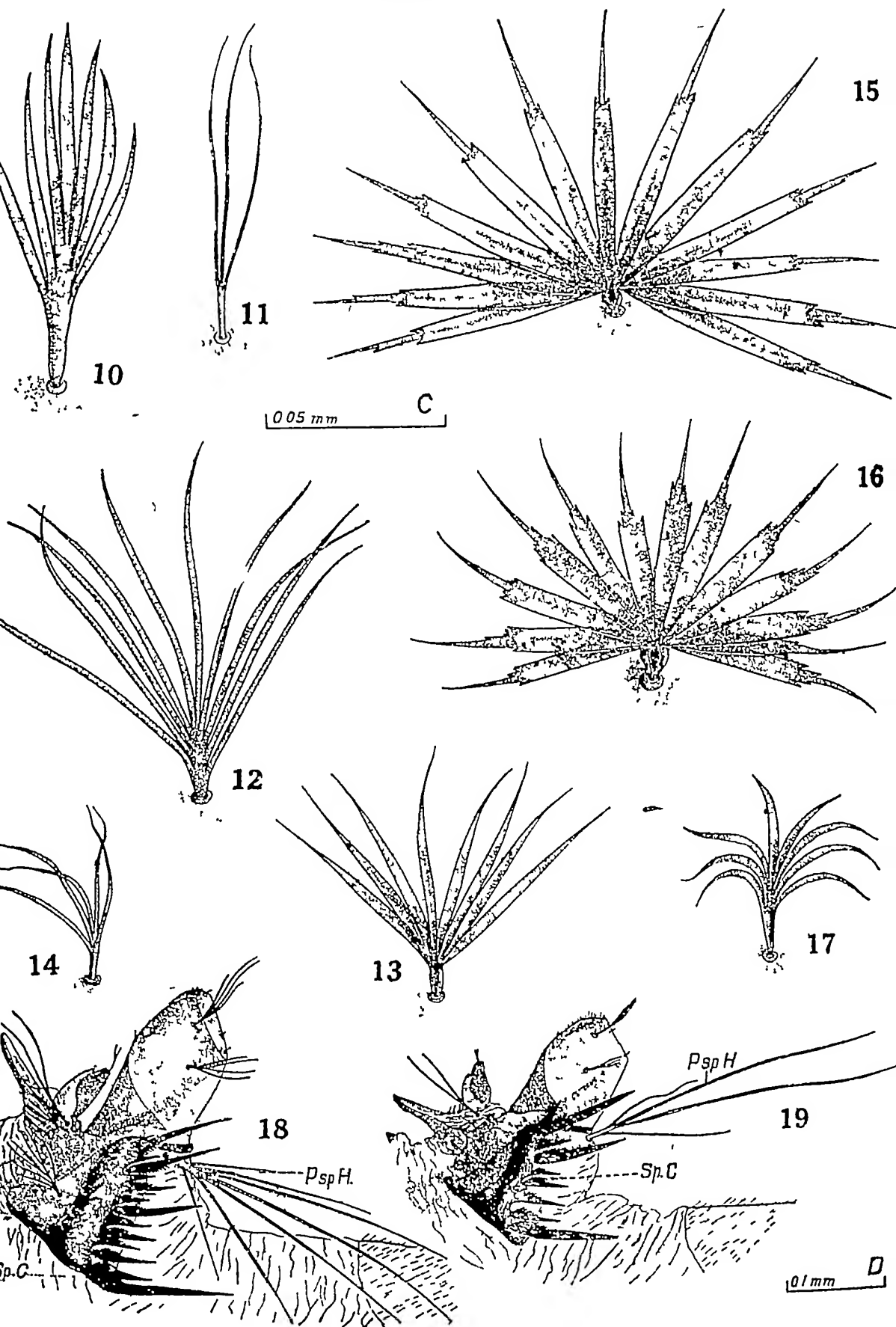
**Abdomen** the first segment has a fairly well-developed palmate hair (Plate L, fig 13) having 7 to 11 leaflets which often show poorly developed filaments. The filaments of the mid-abdominal palmate hairs are very deeply indented at their bases and are comparatively longer than those in *jamesu*, average length of the leaflet being 0.445 mm and that of the filament 0.352 mm.

LARVA OF *A pallidus* THEOBALD 1901

The larva shows a very close resemblance to that of *A fuliginosus* and can be separated with certainty only after dissection.

The head shows the usual dark spots which are sharply defined. **Clypeal hairs** the inner anterior clypeal hairs are about 234  $\mu$ m long and appear to be more strongly chitinised. They bear fine hairs which are more numerous but comparatively shorter than in *A fuliginosus*. These hairs tend to become plumose in distribution on the distal one-fourth of the main hair. **Outer anterior clypeals** are a little longer than half the length of the inner anterior clypeals. They are profusely branched, bearing 15 to 37 branches (average 24). In the majority of specimens, each hair divides distally into two or three main branches which give origin to secondary branches with plumose arrangement, all the branches, however, lie in one plane (horizontal). Each of the **posterior clypeal hairs** bears from 2 to 5 branches (average 3). They divide from the base into two or three branches, which may divide into secondary branches. The **inner occipital hair** is moderately long and divides from its base into 3 to 8 branches (average 5).

PLATE L



which also differs from *A pallidus* in not possessing scales on the thoracic pleuræ

Like the adults, the moults of the final larval instar of these two specimens also show slight but marked differences from the larvæ of *A pallidus*. These differences have been described below \*

**Clypeal hairs** The *inner anterior clypeal hairs* and the fine hairs borne on them are comparatively stronger, the latter also longer in one specimen they are about twice as long as in *pallidus*. Their arrangement on the distal half of the hair is thickly plumose. The *outer clypeal hairs* bear 36 in one and 26 long branches in the other. The main hair splits near its base ( $\frac{1}{8}$ th the length of the hair from its base) into a large number of long branches, two of which are thicker than the rest and bear secondary branches which are plumose in arrangement but lie more or less in the same (horizontal) plane. Each of the *posterior clypeal hairs* divides at its base into 7 to 10 branches with spread out and lie in one plane. The *inner occipital hair* bears 3 to 4 branches.

The *mentum* has 9 teeth as has that of *pallidus*.

Thorax tubercles of the *submedian hairs* are poorly developed and are separate. The innermost hair is comparatively short and much dilated, bearing about 17 branches, the intermediate hair too is much shorter and bears 5 long branches internally and 3 externally. The outermost hair is also short and arises a little apart from the tubercle.

Leaflets of the mid-abdominal palmate hairs are about as long as in *pallidus* and the indentations at the base of the filaments too are as well pronounced but the filament itself is even less than half the length of those found in *pallidus*. In general form the leaflets are quite distinct from those of *A pallidus*.

#### LARVA OF *A. ramsayi* COVELL 1927

Head is dark brown in colour, but the dark spots are not sharply defined. From the depth of the colour, the head appears to be much more strongly chitinated than that of *jamesi* or of *fuliginosus*.

**Clypeal hairs** (Plate XLIX, fig 3) the *inner anteriors* are long, separated at their bases by a distance equal to about one-third the width of the head in this region. They bear a number of fine lateral hairs which are fewer and comparatively shorter than those in the other two species. The *outer anterior clypeals* are about two-thirds the length of the inner anteriors. Each has 2 to 5 short spinous branches on each side. The *posterior clypeal hairs* are short, simple, about one-half to one-third as long as the outer anterior. The branches of the six frontal hairs are comparatively much shorter than those in *jamesi* and *fuliginosus*. The *inner occipital hair* is a little longer than the posterior clypeal hair, and is thin and simple.

The *mentum* (Plate XLIX fig 5) has the usual seven dark teeth. The three teeth on each side of the median tooth are equidistant from one another, unlike the arrangement found in *jamesi* and *fuliginosus*. The *submentum* (Plate XLIX,

\* Since the above description was sent to the press, Dr G. C. Ramsay, Labac Central Hospital, Cachar, has at my request kindly sent to the Central Malaria Bureau six specimens (3 ♂♂ and 3 ♀♀) along with their last larval moults. The examination of these skins from Assam shows that they are similar to the two larvæ from Yellapur, Bombay Presidency.

# KEY TO LETTERING

A Scale for figures 1, 2, 3, 8 and 9  
 B " " " 4—7  
 C " " " 10—17  
 D " " " 18 and 19  
 I A C Internal Anterior Clypeal Hairs  
 O A C Outer " " "  
 P C Posterior Clypeal Hairs  
 P sp H Post-spiracular Hair  
 Sp C Spiracular Comb

*A jamesi*—(contd.)

- (3) Terminal  $3\frac{1}{4}$  hind tarsi invariably completely white. Only a few pale scales at the distal end of the first tarsal segment.
- (4) Dorsum of last two abdominal segments densely clothed with a conspicuous covering of golden hairs and scales.

*A ramsayi*—(contd.)

- Either  $3\frac{1}{4}$  or  $2\frac{3}{4}$  terminal hind tarsal segments white. A distinct white band at the distal end of the first tarsal segment.
- Not so.

## ACKNOWLEDGMENTS

I wish to express my sincere gratitude to Lieut-Colonel S. R. Christophers, F.R.S., Director, Central Research Institute, Kasauli, for his unfailing kindness and for the keen interest he has taken in my work. It was at his suggestion that the present inquiry on the larvæ of the Anopheline mosquitoes of India was started. My thanks are due to Dr. P. Sur, Assistant Surgeon, Malaria Research Laboratory, Krishnagar District, Nadia, Bengal, for sending to the Malaria Bureau, Kasauli, material from which the above study of the larva of *A. ramsayi* has been made.

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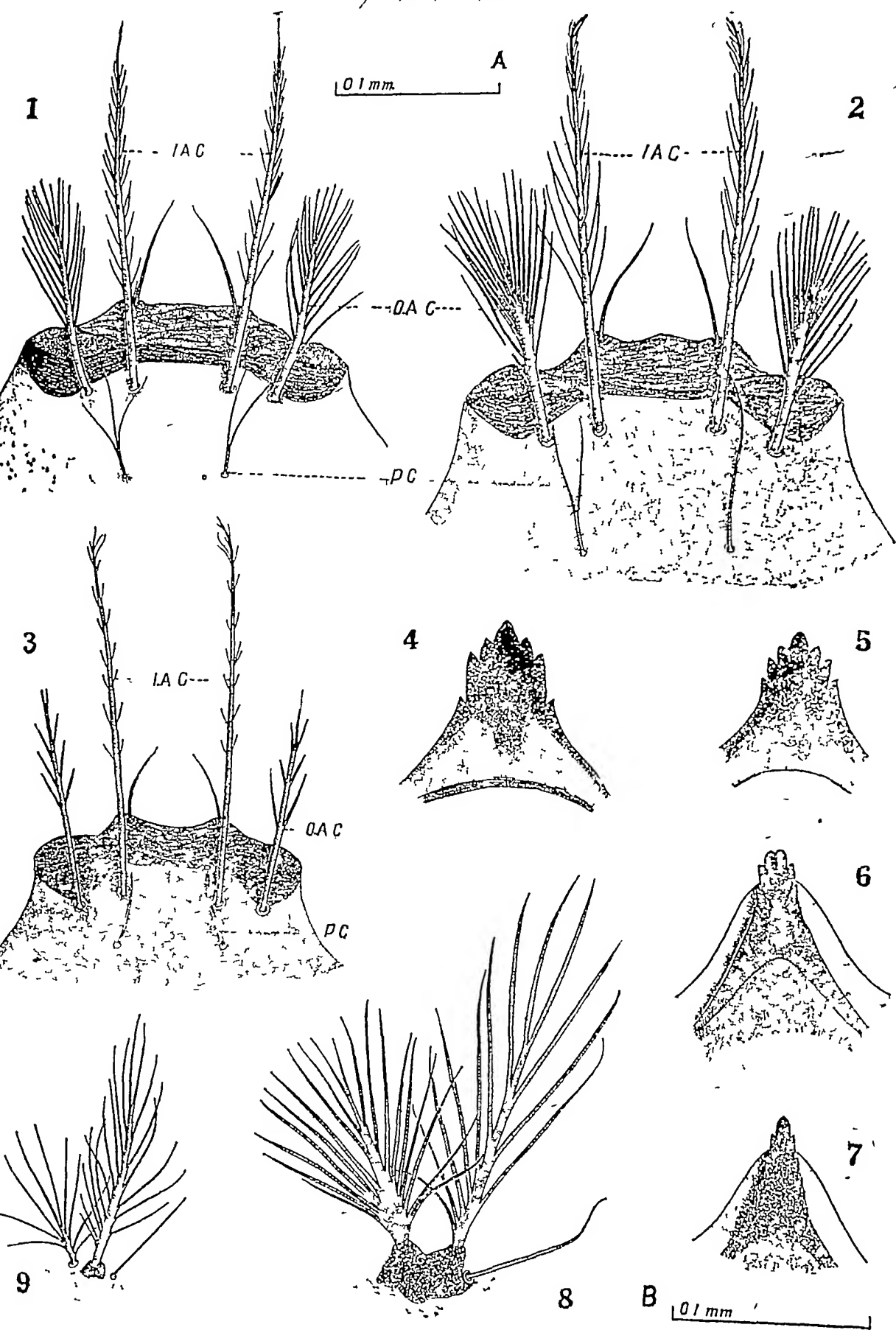
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As will be seen from the accompanying table all the fleas collected were *Xenopsylla cheopis* with the exception of one specimen of *X astia*. The largest number of fleas taken from one rat was 45, and the average number per rat 9

Date	Place	Serial number of rat	Number of fleas found	Number of <i>X cheopis</i>	Number of <i>X astia</i>
2-4-27	Gauhati	1	1	1 female	nil
3-4-27	"	2	1	1 male	nil
3-4-27	"	3	nil	nil	nil
4-4-27	"	4	9	5 males 4 females	nil
4-4-27	"	5	45	16 males 29 females	nil
4-4-27	"	6	nil	nil	nil
6-4-27	"	7	10	7 males 3 females	nil
8-4-27	"	8	1	1 female	nil
10-4-27	"	9 10	34	16 males 18 females	nil
13-4-27	"	11	5	3 males 2 females	nil
16-4-27	"	12	14	5 males 8 females	1 female
18-4-27	"	13	1	1 male	nil
21-4-27	"	14	5	2 males 3 females	nil
23-4-27	"	15	9	2 males 7 females	nil
27-4-27	"	16	13	10 males 3 females	nil
28-4-27	"	17	5	3 males 2 females	nil
		TOTALS	153	152	1
1922	Shillong		62	37 males 25 females	nil

The fleas collected from rats in Shillong in 1922 were all, without exception, *X cheopis*.

Cragg (1920, 1921, 1923) published observations on the distribution of Indian rat fleas based on the examination of over 23,000 specimens collected in various







# EXPLANATION OF PLATE L

- Fig 10 Thoracic palmate hair of right side *A jamesi* Scale C
- „ 11 Thoracic palmate hair of right side *A ramsayi* Scale C
- „ 12 Right palmate hair of 1st abdominal segment *A jamesi* Scale C
- „ 13 Right palmate hair of 1st abdominal segment *A fuliginosus* Scale C
- „ 14 Right palmate hair of 1st abdominal segment *A ramsayi* Scale C
- „ 15 Right palmate hair of 2nd abdominal segment *A jamesi* Scale C
- „ 16 Right palmate hair of 2nd abdominal segment *A fuliginosus* Scale C
- „ 17 Right palmate hair of 2nd abdominal segment *A ramsayi* Scale C
- „ 18 Lateral view of the posterior spiracle *A jamesi* Scale D
- „ 19 Lateral view of the posterior spiracle *A ramsayi* Scale D

a false reaction is secured' (Kolmer, 1923) (6), and again that 'in the hæmolytic system it can be shown that a deficiency of either complement or hæmolysin may be replaced to a certain extent by increasing quantitatively the other' (Park & Williams, 1925) (7)

This belief has been responsible for several variations in the preparation of a hæmolytic system (1) Antiox or antihuman hæmolytic amboceptor with the corresponding red blood cells have been substituted for the antisheep hæmolytic amboceptor and the sheep red blood cells. The substitution yields poorer amboceptor with greater trouble and is not free from objection (2) The natural antisheep hæmolytic amboceptor found in the human serum has been removed prior to the test. This is an added burden on an already cumbersome procedure and is accompanied by other complications (3) The natural antisheep hæmolytic amboceptor and the native complement have been made use of in preparing a hæmolytic system and a hæmolytic index of each serum has been determined. All human sera, however, do not contain the natural antisheep hæmolytic amboceptor which, moreover, when present, may not be sufficient in amount. The complement, again, is a very variable quantity. A test of a serum thus performed would be an individual test involving special care and affording no comparison with the other tests in the batch.

Besides these accurate methods several others not so exactly balanced have been devised. They all depend upon the fact that there exists in the human serum a natural antisheep hæmolytic serum which may (it is feared) vitiate the results when added on to the amboceptor in the usual hæmolytic system (guinea-pig complement, sheep red blood cells and rabbit antisheep hæmolytic amboceptor) and which can itself be used in the hæmolytic system.

It is also believed that 'what is particularly characteristic for this case (a series of experiments—writer) is the fact that the minimal amount of complement is almost reached with a small multiple of the amboceptor unit, and that it does not materially change with a further increase of the amboceptor' (Ehrlich, 1906) (8), again that 'since in the test proper the cells come into contact with a small but variable amount of hæmolytic amboceptor which is present in the human serum, I have always considered it better to sensitize the cells beforehand with a large amount of amboceptor, so that in the test proper the M H D of the complement as ascertained by previous titration will not be affected by the additional amboceptor in the tested sera' (Harrison, 1918) (9), again that the importance of this (natural antisheep amboceptor in human serum—writer) has been over emphasized and the possible influence of natural antihuman hæmolysins in some serums overlooked' (Kolmer, 1923) (10), and again that 'this advantage (using antihuman hæmolytic system and thus avoiding interference by the natural antisheep amboceptor—writer), however, is more than counterbalanced by the disadvantages that exist' (Kilduffe, 1926) (11).

To go into the question of hæmolysis in general or to reconcile the diverse views of the different or even of the same worker is not the intention of the writer at present. His object is only to express a few frankly

# A NOTE ON RAT FLEAS COLLECTED IN ASSAM

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WHILST working with the Kala-azar Commission at Gauhati, Assam, I made a small collection of fleas from rats in April 1927. Owing to the pressure of more important work it was not possible to carry out observations on a large scale, and this note refers to 153 fleas obtained from 17 rats, and to a further small collection of 62 fleas obtained from rats caught in Shillong in 1922, which was kindly given to me by Mr C S Swaminath.

These collections are of interest as the province appears to be practically immune from plague, and little is known regarding the species of fleas occurring on rats in that part of India.

The Director of Public Health, Assam, was good enough to inform me upon enquiry that no case of plague had been reported in Gauhati (Kamrup District) for the past three or four years. In April 1918 a case of plague of the bubonic type was reported from Rangia Bazaar (Kamrup District). The case was imported from Ratangarh, Bikaner, Rajputana, and ended fatally, but no other cases occurred. No mortality amongst rats was detected in the village in which the case occurred.

Gauhati Town lies on the south or left bank of the Brahmaputra river in the north-west of Assam and is an important place of communication between Eastern Bengal, Upper Assam, and Shillong. The last named place is the seat of Government of the Province and is in the Khasia Hills at an elevation of about 5,000 feet.

Rats did not appear to be very plentiful in Gauhati as, although a small reward was offered, only 17 were caught during the month of April. There did not appear to be any local prejudice against the trapping of rats. Six traps were in daily use in various parts of the town. From general appearance, and from measurements taken at the time, all the rats appeared to be *Mus rattus*, with the exception of one (No 7 in the accompanying table). The skin and skull of this specimen were forwarded to the Indian Museum and the identification was kindly supplied to me as probably *Nesokia bengalensis*.

A table showing the typical reactions and giving the results of the whole series

Number	Name	Reaction	IN CASE OF COMPLETE INHIBITION THE EFFECT OF ADDING		Total number of such cases
			Complement (Guinea-pig, excess)	Amboceptor (Rabbit, excess)	
1	A	L			88
2	B	C I	C L	Nil	6
3	C	C I	Nil	C L	3
4	D	C I	C L	P L	1
5	E	C I	Nil	Nil	2

L = Lysis, complete or partial

C L = Complete Lysis

C I = Complete Inhibition

P L = Partial Lysis

Only five cases had no natural antisheep hæmolytic amboceptor. The percentage of the sera containing the amboceptor, in the series, rose to 95 per cent. Other figures are —

1 About 90 per cent (Harrison, 1913) (12).

2 Over 80 per cent (Kolmer, 1923) (13)

3 60 to 80 per cent (Kilduffe, 1926) (14)

Of Partial Inhibition of lysis there were 24 cases

15 lacked complement

5 lacked amboceptor

4 lacked both

Case No 4 in the table may appear to suggest that to some extent complement and amboceptor are interchangeable. This apparent anomaly, however, may (and probably does) depend upon the fact that both natural amboceptor and native complement in man are very much weaker than the rabbit amboceptor and the guinea-pig complement. The native human complement, therefore, while failing with the natural amboceptor will succeed (fully or partially) with the rabbit amboceptor. In the same way the natural amboceptor while failing with the native complement will succeed (fully or partially) with the guinea-pig complement.

The time taken by a serum in effecting a lysis was noted. Observations were recorded for 30 minutes. After 30 minutes no change could be detected.

*Experiment III* Determination of the influence of the natural hæmolytic amboceptor in the human serum on a complement fixation reaction

parts of the country. His papers do not include any reference to Assam, but it may be mentioned that collections received from Rangoon, Burma, contained about equal numbers of *X cheopis* and *X astia*, whilst from Akyab, on the coast of Burma further north, 98·7 per cent of the fleas received were *X astia*. The only collection mentioned as having been received from Bengal was one of 30 fleas from Barrackpore, near Calcutta, 29 of which were *X cheopis* and one *X astia*.

As the specific distribution of *X cheopis* and *X astia* is believed to be an important factor in the epidemiology of plague, it has been thought worth while to publish this note in the hope that others may find time to make further observations in Assam. Collections may be forwarded to the Central Research Institute, Kasauli. Copies of a paper by Sinton (1925) entitled 'The Indian Rat Fleas, with special reference to the identification of the "Plague Fleas,"' which contains instructions for the collection, preservation and preparation of fleas for examination, may be obtained from the institute abovementioned.

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2 An excess of hæmolytic amboceptor, using 10 M H D instead of 5 M H D, does not affect the M H D of the complement

3 A false negative reaction does not result from an excess of the hæmolytic amboceptor

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# ON WASSERMANN REACTION

## I.

### EFFECT OF AN EXCESS OF THE HÆMOLYTIC AMBOCEPTOR ON THE M H D OF THE COMPLEMENT

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It is generally believed that '*little amboceptor + much complement and much amboceptor, + little complement* lead to the formation of the same amount of *complement-amboceptor combination (hæmolytic unit) anchored by the receptors*' (Ehrlich, 1906\*) (1), again that 'within certain limits the quantitative relationship existing between the absolute amount of complement and amboceptor required to produce complete hæmolysis is such that an increase of one factor, say complement, permits the use of a less amount of the other factor, namely, the amboceptor' (Noguchi, 1912) (2), again that 'the same amount of hæmolysis can be produced by one unit of complement and one unit of amboceptor or by 20 units of amboceptor and 0.1 unit of complement, or any other approximate combinations of these two components' (Noguchi, 1912) (3), again that 'within certain limits, the larger the amount of amboceptor with which the cells are sensitized, the smaller the M H D of complement' (Harrison, 1918) (4), again that 'despite all the claims that have been made that the presence of antsheep amboceptor in human serum is a negligible factor in the Wassermann test, the fact remains that in some blood sera there is so much of this amboceptor present that no dependence can be placed upon the results of a Wassermann test with them unless it is first removed' (Craig, 1918) (5), again that 'the important fact to be borne in mind is that a slight decrease in complement may be compensated for by the presence of many units of hæmolysin, so that complete hæmolysis results and

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\*These dates refer to the year of publication of the documents under reference only  
They do not necessarily date the first announcements



cases Italian workers, Cannata (1913-1914), and Vaglio (1914) were similarly successful Mackie (1915) reports the presence of parasites in the peripheral blood in 21 per cent of cases occurring in indigenous Assamese and 64 per cent of cases in a series composed of tea garden coolies More recently, Knowles (1920) found parasites in thirty-three out of seventy-three cases but only after a scrutiny of 682 blood films More recently still Knowles and Gupta (1924) have obtained 67 per cent of successes by the use of a thick film method

A perusal of the literature gives one the impression that the degree of success obtained in the search for parasites in the peripheral blood in kala-azar is largely proportional to the patience as well as the skill of the observer, in other words the amount of material examined, which is what one might expect from the proved almost universal presence of the parasites in the peripheral blood To some extent this has been our own experience, but we differ in this respect from most of the observers mentioned in that we have not found it necessary, in the great majority of cases, to examine large numbers of slides of peripheral blood in any individual case, if the object aimed at is merely to obtain a diagnosis Our figures, also, relate to a somewhat larger series of consecutive proved cases than has been recorded by any of the workers referred to above As the method and extent of the examinations carried out by us have been uniform throughout the series, and as our observations relate to strictly consecutive cases, all proved to be kala-azar by spleen or liver puncture, we feel we are not unjustified in assuming that our results represent a close approximation to those which would be obtained by other workers pursuing similar methods, and should, therefore, be useful as a standard for comparison as to the relative value of other methods

### TECHNIQUE

This, as already stated, was uniform throughout the series Shortt, Barraud, and Craighead (1927) have described the technique employed by them in preparing blood slides with a special view to their examination for the parasites of kala-azar As this method has a very direct application to the subject of the present communication, and was the method employed by us throughout, the description given by the workers referred to is here given 'in extenso' 'A small drop of blood is placed at one end of a slide, a second slide is applied to it, as in making an ordinary blood smear The second slide, as soon as the blood has spread out along its edge, is pushed along the surface of the first with an even motion until the blood is almost exhausted At this point, instead of continuing this motion, as in making an ordinary smear, the second slide is abruptly lifted off, with the result that the blood smear ends in a straight edge stretching transversely across the slide This straight edge is somewhat thicker than the rest of the smear and contains a large percentage of the total white cell content of the drop of blood The white cells in the straight edge are all that it is necessary to examine for the purpose of determining, with a fair degree of accuracy, the presence and numbers of Leishman-Donovan bodies in the peripheral blood' As an invariable routine the terminal edges of four slides, prepared in the way described, were examined for parasites

heterodox opinions on the complement fixation test in syphilis. A hæmolytic system consisting of guinea-pig complement, sheep red cells and antish sheep rabbit hæmolytic amboceptor leaves nothing to be desired. An excess of hæmolytic amboceptor, after a sufficient amount of it has been used for sensitization, does not effect the M H D of the complement. A false negative reaction does not result from an excess of the hæmolytic amboceptor.

These opinions are based on the following work —

*Experiment I* M H D of complement determined with suspensions of red blood cells of the same density but sensitized with different doses of amboceptor

Two sets of tubes were employed to determine the M H D of the complement for the routine Wassermann† Reaction. In set No 1 the usual quantities of the reagents were used. In set No 2 the dose of the hæmolytic amboceptor was doubled (i.e., made 10 M H D instead of 5 M H D).

*Result* The M H D of the complement was found in the same tube in both the sets.

This experiment was repeated for six months, twice a week. The only irregularity observed was an occasional inhibition of lysis (partial) in the case of the suspension sensitized with 10 M H D of amboceptor (Neisser-Wechshberg phenomenon?).

Complement acting in a dilution of less than 1 in 30 and amboceptor in a dilution of less than 1 in 1,000 were rejected. Generally the titres of the complement and the amboceptor were over 1 in 50 and over 1 in 2,000 respectively.

*Experiment II* Detection of the natural antish sheep hæmolytic amboceptor in man

Active sera of 100 apparently normal Indians were tested for hæmolytic activity against sheep cells, the proportion being the same as in a Wassermann Reaction‡.

#### *The Scheme*

Active serum (1 in 5)	1 Vol
Sheep cells (3 per cent)	1 Vol
Saline	2 Vols
	<hr/>
TOTAL	4 Vols
	<hr/>

† The technique used in the Central Research Institute, Kasauli, is that of Method IV recommended by the British Medical Research Committee\*, with the following differences

1 A small volume procedure is followed, using quill tubes (with stirring rods) and Wright's pipettes for measuring volumes

2 An additional tube with 8 M H D of complement is put up. A positive reaction in this tube is termed a + + + reaction

‡ See foot-note under Experiment I

TABLE I

*Showing the details of direct microscopical examination for parasites in slides of peripheral blood in kala-azar*

Total number of cases examined by spleen or liver puncture	Total number proved kala-azar	Total number of kala-azar cases showing parasites in the peripheral blood	Total number of kala-azar cases not showing parasites in the peripheral blood	Percentage showing parasites
1,157	480*	358	97	78.7

\* In a certain number of cases no examination of the peripheral blood was made

#### TOTAL NUMBER OF WHITE CELLS CONTAINING PARASITES OF KALA-AZAR

As previously stated, the only part of the blood slide examined as a routine was the terminal straight edge, four slides being examined in each case. In the 358 cases showing parasites in the peripheral blood there were found a total of 2,168 parasitised white cells. This gives an average per case of observed parasitised cells, of 6.1, and an average per slide of 1.5.

#### THE TYPES OF WHITE CELLS CONTAINING PARASITES OF KALA-AZAR

In order to avoid confusion we have, for the purposes of this description only, placed all the infected cells observed by us under two categories only, viz., polynuclear cells and mononuclear cells. By doing this, we hope to avoid any ambiguity due to the notoriously variable classification of the white cells accepted by various workers. The first category includes only one type of cell, viz., the cell which is commonly called the polymorphonuclear leucocyte. Under the second category are included the large mononuclear leucocyte, the transitional cell, the large lymphocyte, and the endothelial cell. The details of our observations under this head are given in Table II.

TABLE II

*Showing the types of white cells containing parasites and their relative numbers*

Type of cell	Number of cells parasitised	Percentage parasitised
Mononuclear cells	1,086	50.1
Polynuclear cells	1,082	49.9
TOTAL	2,168	100

The same 100 sera were inactivated and their influence on a titrated hæmolytic system was tested in a three-tube test

*The Scheme*

Tubes	1	2	3
Serum, 1 in 5, Vol	1	1	1
Sensitized red cells, as used in a Wassermann reaction, Vol	1	1	1
Complement, 1 Vol, containing M H D	1	$\frac{1}{2}$	$\frac{1}{2}$
Saline Vol	1	1	1

In spite of the natural amboceptor not in one out of the 100 sera could the deficiency of the complement be made good. The difference in the grades of the lysis corresponding to the deficiency was always kept up.

Most of the sets of the three tubes showed more inhibition than the controls without serum.

Agglutinating power of certain sera made the tubes look crystal clear with deposit of the red cells at the bottom.

*Experiment IV* Effect of an increase of hæmolytic amboceptor on the Wassermann Reaction\* of a known syphilitic serum.

As a crucial test +++ , ++± , ++ , +± , + and ± sera as determined in the routine one week, were re-submitted to a double test next week, using the usual and twice as strong a dose of the hæmolytic amboceptor for sensitizing the red blood cells.

To bring out the end point of inhibition of lysis +++ and ++ sera were used in half doses.

Fifty such sera were tested during the last 6 months, not a single serum was found to turn negative from positive because of an increase in the dose of the hæmolytic amboceptor.

The distributor of the reactions over a long period eliminates the influence of the coincident extraneous factors such as temperature, pH of the saline, and peculiarities in the rabbits and the guinea-pigs.

Here it may be mentioned that every ± serum was not always found ± next week even with the usual dose of the amboceptor. Often it was found to have become negative.

This point, however, will be considered again under other headings. It does not appear to be connected with the excess of amboceptor.

SUMMARY

1 A hæmolytic system consisting of guinea-pig complement, sheep red cells and antisheep rabbit hæmolytic amboceptor leaves nothing to be desired.

\* See foot-note under Experiment I

Mononuclear cells—88 parasites, including 19 multiple infections

The largest number of parasites seen in a single cell was 35, seen in an endothelial cell

#### FACTORS INFLUENCING THE PARASITE COUNT

The number of parasites found in the peripheral blood depended, to a considerable extent, on certain conditions affecting the clinical condition of the patient, as will be shortly detailed below

##### (a) Effect of the degree of leucopenia on the parasite count

As all the parasites present in the peripheral circulation are intracellular, except as the result of accidental rupture of the containing cells, the number present in any specimen have a direct relationship to the degree of leucopenia present. Where the latter is severe the number of infected cells per slide is necessarily diminished, but the parasite count may not be diminished in the same proportion. This is due to the fact that in such cases the proportion of mononuclear to polynuclear cells is often increased, and, as the former more frequently exhibit the higher degrees of multiple infection of cells, the balance is to some extent rectified. Nevertheless, it may be said that increasing leucopenia means, on the whole, a diminishing parasite count.

When leucopenia is not a marked feature of a case, the percentage of polynuclear to mononuclear cells is often, although not invariably, increased. In such cases the tendency is for a larger percentage of polynuclear cells to show infections with single parasites and so to raise the total parasite count. The general inference drawn is that a relatively high leucocyte count means a relatively high parasite count. In this connection it may be stated that in practically all cases showing unusually high parasite counts, the degree of leucopenia was relatively slight.

##### (b) Effect of temperature of the patient on the parasite count

As the result of our observations we have been able to make a generalisation to the effect that, other factors being uniform, the presence of a temperature above the normal has an effect in increasing the number of parasites in the peripheral circulation. Whether this phenomenon is 'post hoc' or 'propter hoc' we are not prepared to say, but we have usually found that in cases showing high parasite counts, a relative decrease in temperature, or a drop to normal is almost invariably followed by a drop in the number of parasites circulating in the blood. At the same time it must be noted that many cases during long periods of apyrexia still show continuously the presence of parasites in the peripheral blood.

##### (c) Effect of alimentary symptoms on the parasite count

Patton (1914) has stated that, in his experience, cases showing involvement of the alimentary canal, with resulting diarrhoea or dysentery, often exhibit a relatively larger number of parasites in the peripheral blood. On the whole this has not been our experience, cases with symptoms of involvement of the alimentary canal have not been marked by specially high parasite counts.

##### (d) The parasite count in atypical cases showing little or no enlargement of the spleen

Various observers have noted the occurrence of such cases, where, even in advanced stages of the disease, there is little or no enlargement of the spleen.

# THE FINDING OF PARASITES IN THE PERIPHERAL BLOOD OF KALA-AZAR CASES BY DIRECT MICROSCOPICAL EXAMINATION

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CHRISTOPHERS (1904) was the first to describe the occurrence of *L. donovani* in the peripheral blood of cases of kala-azar. Since that date, although all observers have agreed in confirming this finding, there has been considerable divergence of opinion both with regard to the frequency of its occurrence and with regard to the ease with which the parasites may be found on direct microscopical examination.

As regards the first of these points there can no longer be any ground for difference of opinion, because the use of cultural methods has demonstrated the occurrence of parasites in the peripheral blood in practically all cases of kala-azar. This method of diagnosis was first applied by Mayer and Werner (1914) and independently by Wenyon in the same year. More recently many workers have successfully applied the method to the diagnosis of cases, and, in experienced hands, failures to demonstrate the parasite by this means are infrequent, especially if a considerable number of tubes of the medium used be inoculated. Thus B M Das Gupta (1922) obtained cultures of *L. donovani* from the peripheral blood of nineteen consecutive cases of kala-azar, a result which corresponds with our own experience in this field of observation.

With regard to the second point, the ease with which the parasites present in peripheral blood may be found by direct microscopical methods, there is room for more divergence of opinion, and very varying results have been obtained by different observers, not only as regards the frequency with which the parasites are found in cases of kala-azar, but as regards the amount of observation required to demonstrate them. Thus Donovan (1905, 1909) reports their presence in over 93 per cent of cases while Patton (1907, 1912) obtained them in over 84 per cent of

*(g) Effect of treatment on the parasite count*

This may be stated in a few words. Whatever the form of antimony treatment employed, there is a rapid disappearance of parasites from the peripheral blood. This disappearance is much more rapid in the case of some of the more recently introduced organic compounds of antimony, such as urea stibamine, than in the case of the antimony tartrates. In the former case, parasites usually disappear from the peripheral blood after two or three intravenous inoculations (0.5 gramme in an adult) while in the latter, the disappearance is somewhat slower. This effect of treatment in rapidly sterilising the peripheral blood is one of the most important aspects of all in the treatment of the disease, since it rapidly transforms the kala-azar case, even in the early stages of treatment, from being a great potential danger to others, to a comparatively innocuous individual.

We believe this factor to have had more effect in the fight against the disease in Assam by means of efficient treatment, than the mere cure alone of the thousands of cases treated would have resulted in. To put it even more strongly we believe, taking into consideration the prevalence of the disease in Assam during the years 1917—1927, that were it not for the widespread sterilisation of the peripheral blood due to treatment, the outbreak in this period would have been more widespread and more disastrous than that of the years 1891 to 1901, when vast areas in Assam became waste land owing to the ravages of the disease. A consideration of the figures showing the numbers treated during the last few years in Assam will show the justification for such an assumption. Thus in the years 1924, 1925, and 1926, 48,770, 60,940, and 46,231 cases respectively were treated. All these cases, even those not completely cured, ceased for the time at least to be spreaders of infection, i.e., in the year 1925 alone, there would have been, but for the treatment campaign, 60,940 more sources of infection than there actually were.

A minor point emerging from the disappearance of parasites from the peripheral blood as a result of treatment is that whereas examination of the peripheral blood for parasites, either directly or by culture, is an efficient means of diagnosis, it is of no value whatever as a test of cure after treatment. This is mentioned because we have known certain workers refer to culture from the peripheral blood as a test of cure.

#### VALUE OF MICROSCOPICAL EXAMINATIONS OF THE PERIPHERAL BLOOD IN EXPERIMENTAL WORK

In experimental work in connection with the transmission of kala-azar, the direct microscopical examination of the peripheral blood for parasites has been found to be of the greatest value. It affords a simple, sufficiently accurate and readily available means of selecting those cases which are the most appropriate for use in feeding experiments with blood-sucking insects whose capacity as vectors of the disease it is desired to investigate.

#### SUMMARY

1 The technique of making blood slides for examination for parasites of kala-azar is described.

None of the thick film methods we have investigated, including that advocated by Knowles and Gupta, approach in simplicity, and, in our hands in efficiency, to the method described here. They also possess the universal disadvantage of presenting the white cells in a contracted condition or at least in a nearer approach to a globular form, which, while it may possibly be more like their natural condition, renders the search for parasites much more difficult, and often obscures their identity, as compared with the flattened out white cells of a comparatively thin film.

#### MATERIAL

The total number of cases examined by us with a view to diagnosis by spleen or liver puncture, with a few exceptions otherwise diagnosed, was 1,157. Of these 480 were proved to be cases of kala-azar. The majority of the remainder were cases of malaria, often proved by the finding of malarial parasites in the smears from spleen or liver, especially the latter. A few cases of leukocythæmia were also met with. The number of malarial cases coming for diagnosis and, one fears, often treated as cases of kala-azar at the treatment centres, may possibly be due to the great prevalence of quartan malaria in the districts in Assam where the Kala-Azar Commission was working. The great enlargement of the spleen, which is often a feature of infection with quartan malaria, may readily deceive the clinician in a district where both diseases are prevalent.

Another condition simulating kala-azar was frequently met with by us, which unfortunately pressure of other work has prevented us from ever satisfactorily investigating. This condition was usually met with in young adults who were, with few exceptions, males. The patient presented outwardly a healthy appearance but, on examination, was found to possess an enormously enlarged spleen. This organ varied in size from a downward and right handed extension to the umbilicus to a condition in which it appeared to fill the greater part of the abdomen. The history of the case was always of many years duration, ranging from five or six to fifteen years. In some cases no history of any illness could be elicited while a few cases gave vague and indefinite accounts of occasional fever, but the reason for the appearance of most of the patients before us was confessedly no real illness but merely the discomfort of the enormously enlarged spleen. No malarial parasites were ever found in any of these cases in the peripheral blood, nor did the latter present any other unusual features. Smears from liver and spleen puncture material were equally void of information.

#### RESULTS OF EXAMINATION OF FILMS OF PERIPHERAL BLOOD IN KALA-AZAR

The figures dealt with by us in succeeding sections relate almost entirely to cases examined by us in the course of field work during the years 1925 and 1926. This period is chosen in order to enable us to investigate in some detail a definite and limited number of consecutive cases, but some of the conclusions drawn by us are based on a much larger experience, in support of which we have no definite figures to offer.

In the table given below are set forth in the briefest manner the main facts which have emerged from our investigation, while succeeding sections deal with further points which have been investigated.



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TOTAL NUMBER OF PARASITES OBSERVED, AND THEIR DISTRIBUTION IN  
INDIVIDUAL CASES

In the 358 cases showing parasites in the peripheral blood 3,210 parasites in all were observed. This gives an average of observed parasites per case of 9, and an average per slide of 2.3

As an indication of the distribution in consecutive cases of the parasites which go to make up the total given above, we may give the numbers found in 20 unselected consecutive cases showing parasites in the peripheral blood. These cases showed individually the following numbers of parasites, 8, 11, 5, 8, 5, 7, 6, 7, 4, 8, 10, 1, 3, 14, 8, 2, 2, 11, 3, 18—Total 141

The largest number of parasites seen in four slides in a single case was 174, and the largest number seen in a single slide was 70\*

DISTRIBUTION OF THE KALA-AZAR PARASITES IN THE BLOOD

The slides taken by us were usually made from the finger blood, but in some cases they were made from other parts such as the toe or even ear. There appeared to be no appreciable difference depending on the source of the blood.

As regards the distribution of the parasites in the blood from any single source, this also appeared to be practically uniform. Thus, the number of parasites seen in each of the four slides from any individual case was comparatively uniform, the small differences not being greater than could readily be accounted for by slight differences in the size and shape of the four individual smears. In other words, our general experience supported the statement that the distribution of the parasites in the blood was more or less uniform for any particular case.

DISTRIBUTION OF THE PARASITES IN CELLS IN INDIVIDUAL CASES

This factor showed considerable variation, both as regards the type of parasitised cell most frequent in any individual case, and as regards the unity or multiplicity of the parasites present in infected cells. Thus, some cases showed a great preponderance of infected polynuclear cells over other cells, others showed a preponderance of mononuclear cells infected, while in some the parasites were fairly evenly divided between these two varieties of white cells.

Another difference noted was that some cases showed a great preponderance of infected cells containing only one parasite each, while others showed many cells with multiple infections. In some cases, again, both single and multiple infections were common.

As already stated the largest number of parasites seen in a single slide was 70. Cells containing more than four or five parasites were almost invariably mononuclear cells. As an example of a case showing many parasites the following details are given of the case in which 174 parasites were observed in four slides. These parasites were contained in cells as follows—

Polynuclear cells—86 parasites, including 15 multiple infections

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\* Since writing this we have seen one case in which the number of parasites in four slides was 432 and the largest number in one slide 183. The largest number of parasites in one cell was 41.

oocysts of the gregarine which forms the subject of this communication. The next occasion on which the parasite was encountered was in 1923, also in Assam, when C S Swaminath, working on the kala-azar enquiry of the Indian Research Fund Association in Assam, under the direction of Major H E Shortt, I M S, noticed in dissections of certain sandflies, similar bodies to those previously mentioned by Mackie.

Since the determination by the Indian Kala-azar Commission of the nature of the parasite it has been intermittently studied, as opportunity permitted, and the results of this study are embodied in the description here given of the life-history and morphology of the parasite.

### GENERAL ACCOUNT OF THE PARASITE

*Percentage of flies parasitised* *Monocystis mackiei* was first studied by us in *Phlebotomus argentipes* caught in nature. No accurate record was kept at the time of the percentage of flies parasitised, as other subjects of more importance engrossed the attention of the Commission, but it is certain that the percentage is a high one. It was noted that the number of parasitised flies relative to those which were free of parasites varied from time to time, but it was uncertain to what extent this was dependent on the season or the locality from which the flies were obtained.

Although, then, exact figures cannot be given for flies occurring in nature, it is at least certain that the percentage of flies naturally parasitised in Assam is not under 25 per cent.

The same uncertainty does not exist in the case of flies bred in the laboratory. Here, owing to the crowding together of large numbers of flies, whether adult or larval, within very circumscribed limits, the chances of infection of the larval stages of the fly are increased to an extent which gives an infection rate in the emerging adult flies of 100 per cent. This is the case whether the flies are bred from the adults caught in nature or from flies which were themselves bred in the laboratory. In the former case, the 20 or 30 flies liberated into the oviposition jar, must contain a considerable proportion of infected individuals each liberating hundreds or thousands of oöcysts, so that all the larvæ eventually become infected, while in the latter case, as has already been stated, all the flies are already infected and the larvæ hatching from their eggs invariably become so by ingestion of the oocysts.

*Relationship of stages in the life-history of M mackiei to stages in the life-history of its host*

*Forms of the parasite seen in the adult fly*—In adult flies, the parasite may be encountered in several forms to be considered separately below—

- (a) Large, or adult gregarines
- (b) Various stages in pseudoconjugation and sporogony
- (c) Oocysts

*Large or adult gregarines* (Plate LI, fig 1 and Plate LIV, fig 15)—These forms are in appearance typical acephaline coelomic gregarines and, with the other forms to be mentioned below, will be more fully described in the section on

This clinical form was first described by Dodds Price (1917) and later mentioned by Shortt (1923). Dodds Price makes the statement that in a series of such cases Major Mackie, I.M.S., found parasites in the peripheral blood in 15 out of 23 cases, a much higher percentage than that obtained by him in other cases in Assam. This type of case is characterised by extreme asthenia, and is a severe type of the disease. Mackie (1915) was of the opinion that parasites were more readily found in the peripheral blood of this type of case than in that of cases of the ordinary type. At one time we were inclined to agree with this opinion, but a larger experience now inclines us to believe that there are no sufficiently definite grounds to justify this distinction.

(e) Effect of the stage of the disease on the parasite count

Mackie (1915) has recorded his opinion that the stage of the disease, i.e., whether early or advanced, seems to have little effect on the parasite count so far as the finding of parasites or otherwise is concerned. With this view we are in general agreement with one proviso to the effect that very early cases more frequently give negative findings than cases which are well developed or advanced. Although parasites may be found in such early cases we have never seen one which yielded a high parasite count. There is no such distinction, on the other hand, between cases which are merely well developed cases of kala-azar and those which are very advanced.

(f) Effect of activity of the disease on the parasite count

This we believe to be the most important factor influencing the occurrence of parasites in the peripheral blood. In the course of examination of some thousands of spleen and liver smears, it has become evident to us that *L. donovani* does not always exhibit in the organs the same degree of vital activity as evidenced by active multiplication. Every now and again one comes across a smear which is at once picked out from the rest by the presence of much larger numbers of dividing forms. This we look upon as evidence that, at the particular time this is noted, the disease is making active progress by rapid multiplication of parasites. The result of this is the rapid engorgement of the endothelial cells with parasites, their consequent destruction in large numbers, and the engulfing by new cells of the liberated parasites. Under these conditions it is natural that the phagocytic cells in the blood also should ingest the liberated parasites in greater numbers with a resulting high parasite count in the peripheral blood.

We believe this periodic exacerbation to be a normal occurrence in untreated cases of kala-azar, the disease developing progressively in a series of leaps, rather than exhibiting a general uniform and orderly progress. The periods of exacerbation are accompanied by raised temperature and all the concomitant factors of an active disease process, while the intervening periods are periods of comparative or even absolute apyrexia. If this conception of the established disease be correct, it would explain the increased parasite rate noticed as the result of a raised temperature, since both these factors would be not interdependent, but the like results of a third process, viz., a period of increased activity in the multiplication of *L. donovani* in the tissues.

- (c) Intracellular forms of the parasite
- (d) Large or adult gregarines which may be
  - (1) within the lumen of the gut
  - (2) within the body cavity

*Oocysts*—(Plate LIV, figs 16 and 17) These are readily found in the alimentary canal although the cyst wall appears to be rapidly acted upon by the intestinal fluid and the sporozoites are thus quickly liberated

*Sporozoites*—(Plate LII, fig 7) These may often be found in the gut in very large numbers when the larvæ are derived from heavily infected adults. If the larvæ are fed upon the dead bodies of infected flies, sporozoites may sometimes be seen in the gut in enormous numbers. Typically they are leaf-shaped or resemble a short broad spear point. In heavy infections, large numbers of sporozoites may be seen literally swarming around the epithelial cells of the gut preliminary to their penetration of these. In order to do this, the sporozoites have first to penetrate the peritrophic membrane of the gut but appear to have no difficulty in doing this.

It is certain that large numbers of the sporozoites never complete their life-cycle and must be destroyed in the gut and got rid of in the faeces. If it were not so, the body of the fly could never contain the large number of adult gregarines which would be produced. Although the sporozoites may be found in any part of the alimentary canal of the larva there is a tendency towards the concentration of the parasites in the more posterior parts of the gut.

*Intracellular parasites*—(Plate LII, fig 8) These are present as the result of penetration of epithelial cells of the gut by the sporozoites. As stated in the preceding section, there is a tendency for the sporozoites to collect in the more posterior parts of the intestine and there is consequently a similar concentration of intracellular forms in this situation. The intracellular forms are never seen in large numbers and it seems probable that their growth and subsequent liberation from the epithelial cells, either as a result of their own activity, or as a result of the destruction of these cells, must be a comparatively rapid process. Another reason for the relative paucity of such forms at the time of examination of any individual larva is probably to be found in the fact that, as already mentioned, only a few sporozoites go on to complete their life-cycle. As, however, the larva can become infected at any phase of its existence, it must continually be ingesting fresh oocysts so that by the time it ceases feeding and is ready to pupate, it has repeatedly acquired fresh infections and its final parasite content is the sum of those more fortunate sporozoites which have succeeded in establishing themselves in an intraepithelial habitat.

*Adult gregarines*—(Plate LI, fig 1 and Plate LIV, fig 15) In sections of infected larvæ it is at once seen that the conditions present are very dissimilar to those obtaining in the adult fly. In the case of the latter it was stated that all the gregarines were coelomic in habitat, in the larvæ, on the other hand, the preponderance of the adult forms is seen to be within the lumen of the intestine. In sections of heavily infected specimens, many gregarines may be seen, sometimes lying in parallel rows, separated by the cells of the intestinal canal (Plate LII, fig 9). This double habitat, divided by the wall of the

2 In proved cases of kala-azar, parasites in the peripheral blood can be found by direct microscopical methods in 78.7 per cent of cases without prolonged examination

3 The influence of various factors affecting the parasite count in the peripheral blood is discussed

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show an indistinctness of outline at one part of their circumference, sometimes even amounting to a ragged edge in the smaller and younger individuals, which may represent the portion of the surface layer of the parasite which was most intimately attached to its host cell

#### *Ectoplasm*

*Epicyte*—(Plate LIV, fig 15) This layer is well-developed. It is, of course, owing to its peripheral situation not readily demonstrable in sections of gregarines but may be conveniently studied in isolated whole individuals stained by iron-haematoxylin. If such a preparation be examined it will be found that the cuticle exhibits well-marked longitudinal striations completely encircling the gregarine in the direction of its longest diameter.

Each striation seems to extend the whole length of the gregarine and possibly forms a complete circuit of the body. The striæ lie strictly parallel to one another except at the poles of the parasite and are separated from one another by about  $5\ \mu$  to  $1\ \mu$  varying with the size of the gregarine. Slight undulations on the surface of the body of the gregarine as shown by differences in focus are reflected in corresponding curves in the longitudinal striations.

*Sarcocyste*—This layer is readily demonstrable especially in fresh preparations where it appears as a clear hyaline outline to the parasite. In stained preparations, whether of the whole parasite or of sections, it is less in evidence.

*Myocyte*—This layer, if it exists, is not easily demonstrable. In some stained preparations, however, an indication of longitudinal folds in the superficial structure of the parasite is seen, and these can be brought more fully into evidence by sliding the cover-slip so as to bring them into greater prominence by some degree of torsion of the parasite by rolling. In a few preparations we have also seen some scanty transverse striæ which may also belong to this layer. The longitudinal folds mentioned in this section are not to be confused with the longitudinal striæ of the epicyte.

*Endoplasm*—In the fresh condition the adult gregarine has a distinctly opaque ground glass appearance. This is possibly due to the markedly granular nature of the endoplasm. The nucleus and even the karyosome can be very distinctly seen. Cytoplasmic inclusions, when present, are also easily discernible. In specimens stained with iron-haematoxylin, the cytoplasm is seen to be very markedly granular. This appearance is fairly uniform throughout the substance of the cytoplasm but is less marked in the neighbourhood of the nucleus and of any cytoplasmic inclusions which may be present. The result of this rarefaction near the nucleus or the cytoplasmic inclusions presents, in optical section, the appearance of a pale coloured halo around the nucleus or other body. This phenomenon is not an uncommon one in other protozoa and on the assumption of a very fluid constitution of the endoplasm, is probably due to the effect on the granular contents of the endoplasm of surface tension at the circumferences of the intra-cytoplasmic bodies.

The question as to whether the granular appearance seen in such fixed and stained preparations represents a close approximation to the granular structure which is actually present in the living individual we consider to be more than doubtful. The statement often made in textbooks that wet-fixed preparations of

# MONOCYSTIS MACKIEI N SP PARASITIC IN PHLEBOTOMUS ARGENTIPES, ANN AND BRUN

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## TABLE OF CONTENTS

### INTRODUCTION—

#### GENERAL ACCOUNT OF THE PARASITE—

Percentage of flies parasitised

Relationship of stages in the life-history of *M. mackiei* to stages in the life-history of its host

#### MORPHOLOGY—

Adult gregarine.

The young intracellular gregarine

The newly-released sporozoites in the alimentary canal of the larvæ

The oocysts

Various stages in pseudoconjugation and sporogony

Sporogony

#### LIFE-CYCLE—

## INTRODUCTION

THE gregarine parasite which forms the subject of this description was first recognised as a gregarine by the Indian Kala-azar Commission, then under the direction of Lt-Col S R Christophers, CIE, OBE, FRS, I M S, and was mentioned in the departmental reports of that body in October 1924. It was subsequently recorded by the ancillary kala-azar enquiry at the Calcutta School of Tropical Medicine in their departmental quarterly reports. The first record, however, of the parasite was almost certainly that of Mackie (1915) when working on kala-azar in Assam in 1914. He described certain bodies seen by him in *Phlebotomus* sp as 'capsulated and hyalin-like in fresh preparation—oval in shape with slight polar protrusion, resistant to stains except in the case of some individuals which revealed a new marked azurophilic nuclear figuring'. The description of these bodies corresponds with the appearance of the oocysts of the parasite as seen in the fresh state in dissections of *Phlebotomus argentipes*, and the species of *Phlebotomus* concerned was said to contain mammalian blood. As the only species feeding on mammalian blood likely to be commonly encountered in the area where Mackie was working is *Phlebotomus argentipes*, there is the strongest presumption that the bodies reported by him were the



karyosome They are bounded by a very distinct and perfectly circular dark-coloured outline and the space within them appears to be of a lighter shade than the ground colour of the karyosome

Their significance is not known, but they appear to be constantly present and possibly represent vacuoles in the karyosome

*Cytoplasmic inclusions* — (Plate LIV, fig 15) In most stained preparations there are present one or more bodies more darkly stained than the ground-work of the cytoplasm These bodies are usually more or less spherical and, while varying in size, are usually about  $4\ \mu$  to  $5\ \mu$  in diameter They present no characteristic structural features and give the appearance of being mere local concentrations of the granules which are elsewhere more evenly distributed They are constant neither in number nor in their position in the body of the parasite and probably are not essential structures When present, they exhibit the 'halo' effect already mentioned when describing the nucleus

In the fresh state, the body of the gregarine is noticeably granular in appearance This granular structure causes the parasite to be markedly opaque and gives it a characteristic ground-glass appearance when seen by reflected light When seen by transmitted light, on the other hand, the granules appear to be light brown or yellowish in colour The nature of these granules is uncertain but the fact that the fresh parasites take up a uniformly intense stain in iodine, points to the likelihood that they are composed of glycogen or paraglycogen

*The young intracellular gregarine* — (Plate LII, fig 8) This is only met with in the larval stages of the fly In its earliest form it tends to be spherical in shape and is placed between the nucleus of the gut cell and its free margin in the lumen of the gut As the gregarine grows it tends to become more or less triangular in shape, the angles being rounded and the base of the triangle being directed towards the lumen of the gut, while the apex forms the point of attachment of the parasite to its host cell There is no special organ of attachment

*Size* — This naturally varies with the stage which the gregarine has reached By the time the gregarine has completely destroyed the host cell and is about to become free, it measures about  $23\ 4\ \mu$  in its longest diameter

*Shape* — This has already been dealt with in a previous section

*Cytoplasm* — The description given of the cytoplasm of the adult holds good for the intracellular forms with a few qualifications We have not been able to demonstrate the myocyte in intracellular forms and it is possible that it may not be developed at this stage as there can be no necessity for any movements of the parasite The endoplasm is granular like that of the adult but the granular structure is somewhat finer in texture The nucleus, with its single karyosome, appears to be identical in structure with that of the adult, differing only in its smaller size It bears about the same relation in size to the body of the parasite as in the adult

*The newly-released sporozoits in the alimentary canal of the larva* — (Plate LII, fig 7)

*Size* — The sporozoit when released from the oocyst measures about  $4\ 8\ \mu$  in length and  $1\ 8\ \mu$  in greatest breadth

**morphology** They are distributed throughout the body cavity of the insect but are never found within the lumen of any part of the alimentary canal. The largest numbers are usually found in the abdomen and they appear to cluster together especially in the neighbourhood of the internal reproductive organs. In the thorax of the insect, most of the parasites are found on the ventral aspect, but any situation may be invaded and frequently considerable numbers of parasites may be found occupying even the legs of the fly throughout a great part of the length of the femora. On one occasion a parasite was seen occupying a position on the dorsal aspect of the head and indenting the supra-oesophageal ganglionic mass.

*Stages in pseudoconjugation and sporogony*—(Plates LI and LIII) All stages in the evolution of the parasite, from the preliminary association of two adult gregarines to the final production of oocysts containing eight sporozoites, are to be seen in the adult fly. The two adult gregarines which approach one another in the preliminary stage of pseudoconjugation are somewhat elongated and apparently identical in appearance. As their contiguous margins are applied to one another, the plane of junction becomes flattened so as to appear a practically straight line in optical section. A slight indentation or sulcus in the general spherical or subspherical form of the associated parasites marks the line of junction of the two individuals. The cyst wall which forms around the two gametocytes is delicate and little in evidence at this stage. As the successive stages in pseudoconjugation, commencing with the division of the nucleus in each individual and ending with the production of male and female gametes, are proceeding the containing cyst wall (gametocyst) becomes more clearly defined but is never at any stage other than a comparatively delicate structure. The process of pseudoconjugation, so far as we have seen, always takes place in the portion of the coelomic cavity contained within the abdomen and more especially in its more posterior two-thirds.

*Oocysts*—(Plate LIV, figs 16 and 17) These, the final product of sporogony, have a broad spindle shape and may be seen either still contained within the gametocysts in which they were produced, or they may lie apparently free within the body cavity. They are often present in such large numbers as to monopolise at least two-thirds of the entire space within the abdomen of the fly (Plate LIII, fig. 11).

*Forms of the parasite seen in the pupal stage of the fly*—Considerable numbers of pupæ, representing stages from those recently formed to those which were nearly ready for the emergence of the fly, have been examined. The only stage of the gregarine encountered in any of these has been the adult form. In the pupa, as in the adult, it is often present, as would necessarily be the case, in large numbers, and the individuals may be located in any part of the body cavity but only rarely in the most anterior parts near the head.

*Forms of the parasite seen in the larval stage of the fly*—In the larvæ, the parasite may be encountered in four forms, viz —

- (a) Oöcysts which have been newly ingested and still contain the sporozoites
- (b) Sporozoites newly liberated from the oocysts and attacking the epithelial cells of the gut

Under favourable conditions of lighting this mass may be seen to be composed of numerous sporozoites but in such a preparation they cannot confidently be counted. In specimens stained by iron-haematoxylin, the structure is readily made out. In this connection it should be pointed out that fixation of these bodies is somewhat difficult owing to the resistant capsule and special methods must be utilised. In such a preparation it is at once apparent that the oocyst contains eight sporozoites applied closely to one another in a tight bundle. Although their general arrangement in the oocyst is such that their long axis is in the long axis of the oocyst, yet they are placed as it were 'head to tail' if the broader end may be called the 'head' and the narrower the 'tail'. This makes for economy of space and, since the nuclei are not quite centrally placed in the sporozoites, results in the nuclei being fairly evenly distributed over the oocyst instead of occurring in an equatorial band as they would have done had the sporozoites all been similarly orientated. The contained sporozoites, although somewhat more attenuated owing to pressure, are almost identical in structure with the free forms described in the preceding section. The nuclei of the sporozoites are usually of the first type described, viz., with two polar masses of chromatin, but the other types are also seen. When the oocyst ruptures in the alimentary canal of the larval sandfly this rupture appears to take place at one or both poles of the oocyst at the position occupied by the projection described. The sporozoites always emerge from the openings so made and, as this happens, the oocyst wall becomes crumpled and folded like a half-inflated rubber ball as it loses the support of its previous contents.

#### *Various stages in pseudoconjugation and sporogony*

*The newly formed gametocyst*—(Plate LI, figs 2 and 3) The two gregarines which are going to associate in pseudoconjugation are usually somewhat elongated and shaped like crescents with blunt extremities.

Instead of the inner margin of the crescent being concave, it may be nearly straight or even slightly convex. The individuals come into close apposition with their less convex surfaces adjoining. As they approximate more closely the plane of junction of the two gregarines comes to be practically flat and is represented in optical section as a straight line. A definite cyst, the gametocyst, is now formed enclosing the associated individuals.

*Size*—The size of the gametocysts shows considerable variation. In longest diameter they may vary between extremes of  $152.1\ \mu$  and  $66.3\ \mu$ .

*Shape*—The shape of the gametocyst is spherical or a broad oval. Unlike the free gregarines, which show much plasticity and can be altered in shape by the pressure of surrounding objects, the gametocysts appear to be rigid structures and maintain their spherical form wherever they may be situated.

*Structure*—The individuals associating appear to be identical. No difference between male and female gametocyte is discernible. In structure of the cytoplasm and nucleus they appear to differ in no way from the typical adult gregarine as described in a previous section.

*Division of the nuclei of the gametocytes*—Soon after formation of the gametocyst the nucleus in each individual divides. Not many forms have been

alimentary canal can be similarly demonstrated in fresh preparations from dissections of larvæ. The adult gregarines lying within the gut appear to be present not among the intestinal contents themselves, but to be lying between the gut cells and the peritrophic membrane.

### MORPHOLOGY

The multiplicity of forms met with in parasites of the order Eugregarina, representing successive stages in the life-history, are, on the whole, remarkably uniform throughout the order. This same multiplicity, however, renders a description of the morphological characters somewhat tedious since each phase in the life-history requires its own separate description. For this reason only those stages in the life-history are here considered which represent definite steps from one form to another, and intermediate forms such as the growing gametocyte, which differs only in size from the fully-grown adult, have as far as possible been omitted from the description. The stages to be considered from the morphological point of view are enumerated below and will be treated in detail seriatim. They are —

- (a) The adult gregarine
- (b) The young intracellular gregarine
- (c) The newly released sporozoites in the alimentary canal of the larva
- (d) The oocysts
- (e) Various stages in pseudoconjugation and sporogony

*The adult gregarine* (Plate LI, fig 1 and Plate LIV, fig 15)

*Size*—The adult gregarine varies considerably in size if this is computed simply by measuring the greatest length and breadth of an individual. The reason for this is that the parasite, besides having the power of movement whereby it can voluntarily lengthen and shorten its body, is also considerably compressible, so that it may by the pressure of surrounding bodies be greatly distorted in outline. An average individual in a state of quiescence, and undistorted by its surroundings, measures about  $101.4 \mu$  in length and  $78 \mu$  in greatest breadth. Larger and smaller individuals are common apart from the disturbing factors mentioned.

*Shape*—Apart from the effects of voluntary movements and of pressure from surrounding objects the natural form of isolated adult gregarines in the living condition is pear-shaped. The narrow end of the pear contains less granular material than the rest of the body and may even be almost completely hyaline. In specimens wet-fixed and stained, the parasite is more or less egg-shaped, i.e., the general outline is ovoid, but, as a rule, one pole is somewhat broader than the other. The pear-shape is preserved if the specimens are first fixed in vapour of osmic acid.

*Consistency of body*—The body of the parasite is probably semi-fluid or at least gelatinous in consistency. This is evidenced by its ready plasticity which allows it to conform to the irregularities of its general surroundings. Similarly, two or more gregarines compressed together assume a flattened shape at the planes of junction with each other (Plate LIII, fig 11).

*Organs of attachment*—No definite organ for attaching the gregarines to their cell hosts can be demonstrated, but the majority of isolated individuals

5 As the chromosomes reach the extremities of the spindle they again break up into comparatively large granules. The spindle is now considerably attenuated in its middle (Plate LIV, fig 18f)

6 The spindle parts in the middle and for a short time a part of it may be still visible in the form of a comet's tail attached to each of the new nuclei (Plate LIV, fig 18g)

7 The new nuclei acquire a nuclear membrane as a very temporary measure but rapidly proceed to repeat the process of division (Plate LIV, fig 18h)

### *Sporogony*

*Gametes* — (Plate LI, fig 6a) As soon as the multiplication of nuclei is complete by which time there are present in the gametocyst some hundreds of nuclei, there is an organisation of the cytoplasm around the nuclei to form an equal number of fully developed gametes. These gametes, whether derived from male or female gametocyte, appear to be identical in structure and size and seem to be evenly distributed throughout the substance of the gametocyst. The nucleus in each is subspherical and has a nuclear membrane. The chromatin is in the form of several large masses distributed irregularly on the linen network. The cytoplasm is vacuolar in appearance.

*Zygotes* — (Plate LI, fig 6b) The male and female gametes appear to unite as soon as they are fully developed, and we have not been able fully to follow the process in detail. The resulting zygotes now rapidly secrete around themselves an impermeable membrane and assume a broad spindle shape similar to the oocysts already described. We have been unable to obtain enough material exactly at this stage to enable us to describe the nucleus of the zygote. The reason for this appears to be that the nucleus of the zygote without any resting stage proceeds immediately to divide. It loses its nuclear membrane, if such was present, and is seen to be composed of an aggregation of large granules of chromatin. A spindle is formed in the cytoplasm but, as in the case of previous divisions, although the spindle may be well seen no definite centrosomes can be distinguished. The granules of chromatin separate and proceed to the two poles of the spindle which is usually disposed somewhat diagonally to the long axis of the zygote. The division appears to be always an unequal one, the chromatin at one pole of the spindle representing only a fraction of the whole. This smaller mass, sometimes apparently represented by one large dot only, appears to be got rid of in some way as it does not, at a later stage, appear to be present. It would appear, therefore, that this act of division representing the first division of the zygote is definitely a meiotic division. The remaining nucleus in the zygote, an aggregation of large granules of chromatin, does not appear to acquire any definite nuclear membrane but proceeds at once to a rapid series of divisions ending in the production of eight sporozoites in what is now called the oocyst. The first of these divisions is readily seen and is definitely by mitosis, the process being similar to that already described for the zygote at its reduction division except that the division is now equal. The two subsequent acts of division to produce eight sporozoites are less easily studied on account of the limited space within which they occur, but there is no reason to believe that they differ in any

issues give a true picture of their actual constitution and structure, is demonstrably false. It is true that where an organism or cell has certain comparatively rigid structures, whether of endoplasmic or ectoplasmic origin, structures such as flagella, axostyles, cilia and membranes, these structures are preserved in wet-fixed preparations, in a condition closely similar to their condition in the living organism. When, however, the substance to be fixed is the fluid or semi-fluid substance of endoplasm the same faithful preservation of form and structure cannot be achieved. In the case which we are considering, the endoplasm of the adult gregarine, the granular structure in the form revealed in wet-fixed preparations stained with iron-haematoxylin is almost certainly an artefact. A very similar structure can be produced by the fixation of various organic fluid or semi-fluid substances which in their fresh condition have certainly no granular structure. Thus a picture very similar to the endoplasm of the gregarine can be obtained by fixing such a fluid as the water of condensation of NNN medium where the fresh fluid is certainly not granular in structure. The granular structure obtained in the gregarine in fixed preparations, therefore, probably bears no close resemblance to the actual granular constitution or structure of the endoplasm, but is the result of the coagulation of its more fluid constituents which, in the process, become aggregated in small masses which give the characteristic granular appearance.

*Nucleus*—(Plate LI, fig 1 and Plate LIV, fig 15) The nucleus of the adult gregarine is a large spherical or subspherical body measuring about  $30\ \mu$  in its longest diameter. Its position in the body is usually eccentric. There is a distinct and well-marked nuclear membrane. If an iron-haematoxylin preparation be moderately decolourised the nucleus is presented as a much darker structure than the cytoplasm. Placed eccentrically is a large densely-staining spherical or subspherical karyosome measuring 8 to  $10\ \mu$  in diameter, with a 'halo' surrounding it, similar to that described around the nucleus. The general appearance is such that, if one were to imagine the body of the gregarine removed, the nucleus itself could easily be mistaken for a complete gregarine, the karyosome representing the nucleus. This will at once become evident from a glance at Plate LI, fig 1, representing an adult gregarine. The contents within the nuclear membrane are more densely granular than the cytoplasm itself but otherwise present a not dissimilar appearance. The nature of these granules is not known but they do not appear to be chromatinic in nature. In a specimen differentiated to the moderate degree mentioned the karyosome, which is more or less spherical, is a densely black eccentric mass showing no details of structure.

If the differentiation of the gregarine be carried further, the interior of the nucleus may become almost clear while there is presented the appearance of a very distinct and well-marked nuclear membrane. In the clear area within this there may be a few granules which have not the appearance typical of chromatin. There does not appear to be any tendency to aggregation of chromatin on the nuclear membrane, most of it being concentrated in the karyosome. If differentiation is carried far enough the karyosome is found to present a more or less uniform dark colouration but is seen to contain two, three, or more spherical bodies  $1$  to  $2\ \mu$  in diameter. The bodies appear to occupy any position in the

sections, to be even more numerous within the lumen of the gut than in the coelomic cavity. Whether in the pupal stage the lumen-dwelling gregarines are destroyed, or whether they then gain the coelomic cavity we are unable to say. The former alternative seems unlikely, and we have never seen any evidence of the destruction of gregarines within the gut in the pupal stage of the fly. On the other hand, their passage from gut to coelomic cavity is difficult to explain even if it occurs during the reconstitution of anatomical elements involved in the destruction of the larval gut and the building-up of the adult gut. If there were first a complete breaking down of the larval gut before the reconstitution of that of the adult one could understand the liberation of the contained parasites into the coelomic cavity, but this, so far as we are aware, does not occur. If, on the other hand, the adult gut is formed around the larval gut, one would expect the gregarines to retain their position inside its lumen. We feel sure that the explanation of the phenomenon requires only a competent knowledge of the processes involved in the reconstitution of anatomical elements taking place in the earliest stages of pupation.

The emergence of the adult fly initiates fresh activity on the part of the gregarines. The adult gregarines rapidly proceed to associate in pairs, male and female (pseudoconjugation), within the body cavity and each associated pair becomes surrounded by a cyst wall the gametocyst. This association takes place irrespective of whether the fly feeds or not. The individuals within the gametocyst do not fuse but remain distinct entities.

Each proceeds by a process of mitosis to increase the number of its nuclei until there are some hundreds present within the gametocyst. When the sum of nuclear divisions is complete there is a concentration of cytoplasm around each nucleus to form an equal number of separate bodies, the male and female gametes. Those which are present on the surface of the gametocysts cause each a slight local bulging of the surface to give it a tuberculated contour somewhat like a raspberry, although the bulges are less marked than in the fruit. The male and female gametes are identical in appearance and they now unite in pairs to form zygotes. Each zygote, the whole still contained within the original gametocyst, now becomes surrounded by a tough membrane and assumes the shape of a broad spindle. Within this membrane, the zygote proceeds to divide by mitosis three times, with the production of eight sporozoites. The resultant body is the oocyst. This retains the broad spindle-shaped contour with small terminal knobs and has an extremely resistant capsule. The oocysts are still contained within the wall of the original gametocyst. As the process just described is repeated many times in the same fly there come to be present numbers of these cysts containing fully developed oocysts. These press upon one another and are also subjected to great pressure by the mass of developing eggs in the ovary of the fly. The result of this mutual compression appears to be the degeneration of the dividing walls between the common oviduct and the oocyst-containing cysts, with the resulting invasion of the oviduct by masses of fully-developed oocysts. These may in cases be so numerous as to fill at least two-thirds of the whole abdomen and, at oviposition they may be seen to emerge in hundreds with each egg laid, thereby providing for the infection of the next generation of flies.

*Shape*—The shape assumed as soon as it is a free individual is very constant and resembles a conventional leaf-shape. It may also be likened to a short broad spear head. There is a definite periplast which serves to preserve the typical outline of the parasite.

*Cytoplasm*—There is no evidence of any differentiation into layers as in the case of the adult gregarine. Even if present, such layers would be difficult to demonstrate on account of the small size of the bodies. The cytoplasm in stained preparations is generally finely reticular or vacuolar in structure and closely resembles that seen in *Leishmania* or *Leptomonas* prepared in a similar manner. A few granules are sometimes seen but appear to be accidental productions and may not be really intracellular.

*Nucleus*—The nucleus is a characteristic structure and does not resemble the nucleus of the adult. It is usually ovoid, of large size, occupying from one-third to one-quarter the length of the body and generally eccentric in position, being placed somewhat nearer the broader end of the body.

It may at times be subspherical in shape. The greater diameter is usually in the long axis of the parasite but it may be diagonally placed. There appears to be a distinct nuclear membrane staining a darker colour than the ground colour of the nucleus. There is no indication, as in the adult, of the constant presence of one definite karyosome. On the contrary, there are characteristically present two or more masses which appear to be true chromatin masses. These masses vary in their disposition in the nucleus, but four variations are commonly found.

(a) There may be a comparatively large mass of chromatin at each pole of the somewhat elongated nucleus.

(b) Similar to (a) but with the two chromatin masses joined by a central rod of chromatin.

(c) Where there are somewhat smaller masses of chromatin similarly placed, but accompanied by still smaller masses placed on the nuclear membrane between them, one on either side.

(d) Where four approximately equal masses of chromatin are placed on the circumference of the nuclear membrane at approximately equal distances.

While these dispositions of the chromatin appears to be those most commonly seen, there are necessarily many variations caused by different orientations of the parasite. The important feature, however, is that instead of one definite karyosome as in the nucleus of the adult, no such body is characteristically present in the free sporozoites, and the chromatin of the nucleus is typically divided up into two or more separate bodies.

*The oocysts*—(Plate LIV, figs 16 and 17)

*Size*—The oocyst when released from the original gametocyst measures about  $9.6 \mu$  in length and  $5.8 \mu$  in greatest breadth.

*Shape*—The shape is that of a broad spindle. Instead of the two poles coming to a point, they terminate in knob-like projections. The effect is to produce an almost exact resemblance to the egg of the common intestinal worm *Trichuris trichiura*.

*Structure*—In fresh preparations, the oocyst is seen to be composed of a surrounding tough transparent membrane enclosing a highly refractile mass



# EXPLANATION OF PLATE LI

- Fig 1 Adult gregarine
- " 2 { Earliest stages of pseudoconjugation—gametocytes in apposition
- " 3 {
- " 4 Early stage of nuclear multiplication in gametocyst—8 nuclei present
- " 5 Later stage of nuclear multiplication in gametocyst
- " 6 (a) Nuclear multiplication in gametocyst completed—hundreds of gametes present
- (b) Formation of zygotes in gametocyst

seen at this particular stage where the first nuclear division is occurring, but, in the cases observed, the division appears to be a very simple process. No evidence of the complicated mitotic division described for some gregarines was seen but it is possible that the study of more ample material at this stage would have revealed some such process. This seems the more probable since later stages in division, when a considerable number of nuclei are present in each individual, are certainly mitotic in character.

*The gametocyst when each gregarine contains four to eight nuclei*—(Plate LI, fig 4) Once division commences within the gametocyst it continues with extreme rapidity so that nuclei in a state of complete rest are seldom if ever seen.

The nuclei at this stage appear, as they do throughout, to be identical in the two individuals. The typical appearance of the newly formed nuclei is as follows. The nucleus is spherical or subspherical and measures  $4.1\mu$  in diameter. There is a very distinct nuclear membrane on which there are arranged numerous masses of chromatin. These vary in number from two or three only up to a condition where they form almost a complete ring, a condition in which the individual masses are small in size. Occupying a central or slightly eccentric position is a single distinct karyosome. Between this karyosome and the periphery of the nucleus, numerous masses of chromatin are scattered over the linin network. In some cases the masses on the nuclear membrane appear to be connected across the nucleus by delicate rods of chromatin.

*The gametocyst in the later stages of division of the nuclei of the gametocytes*—(Plates LI and LIII, figs 5, 12, 13 and 14) When the nuclei in each individual contained in the gametocyst have reached a large number, the method of division becomes more evident, both because there are so many dividing nuclei to examine and because the cytoplasm of the two individuals seems to become somewhat more finely granular and to stain less densely, and thus the finer details of nuclear division are less apt to be obscured. If a gametocyst in this stage be examined, the dividing plane between the two contained individuals will still be evident, but no differences are noticeable in the characters of their respective nuclei. The majority of the nuclei will be found in some stage of division. These stages represent a definite mitosis and the sequence now given appears to be the order in which the stages occur (Plate LIV, fig 18).

1 The spherical nucleus described in the last section (Plate LIV, fig 18a) loses its nuclear membrane and the chromatin is represented by numerous fairly large masses. No definite centrosome is visible (Plate LIV, fig 18b).

2 The chromatin tends to assume the form of short thick rods irregularly arranged. This presumably represents the formation of chromosomes (Plate LIV, fig 18c).

3 These chromosomes split and there is the formation of an equatorial zone of chromosomes. In the meantime a distinct spindle has been forming although no definite centrosomes are at any time visible (Plate LIV, fig 18d).

4 The chromosomes separate from one another, presumably after splitting and proceed towards the two poles of the spindle, which is now very distinct and may measure as much as 8 to 10  $\mu$  between its two extremities (Plate LIV, fig. 18e).



particular from the first division. The resulting oocyst with its eight sporozoites has already been described.

### LIFE-CYCLE

The preceding description has already touched upon most of the phases seen in the life-cycle of *Monocystis mackiei* and it only remains to marshal and connect in their proper sequence these successive phases. For this purpose it seems most convenient to commence at the stage where the larval *Phlebotomus argentipes* acquires its infection.

The adult sandfly in oviposition deposits its eggs scattered singly over a small area. Each egg deposited is accompanied by what can only be described as a 'shower' of gregarine oocysts. These oocysts are lying in the common oviduct of the fly, and the impulse which drives out the eggs ejects also the parasites. When the sandfly larva emerges from the egg, it is at first comparatively quiescent and does not move far from the place where it hatched out. It is necessarily surrounded by the oocysts of the gregarine, which, owing to their resistant envelopes, have remained unaltered while the sandfly egg was developing. The first food taken by the larva is, therefore, accompanied into the intestine by numbers of gregarine oöcysts. These oöcysts, reacting to their new environment, discharge their contained sporozoites, in the manner already described, among the intestinal contents. The sporozoites now appear to be irresistibly attracted to the gut wall, make their way through the peritrophic membrane and literally swarm around the epithelial cells of the gut wall. The more posterior parts of the gut are especially the seat of this invasion. A certain number of the sporozoites succeed in invading the epithelial cells but the majority presumably fail to do so. Those which have thus acquired an intracellular habitat increase in size at the expense of their host cells, to one part of which they appear to be attached, until the host cells are completely destroyed and represented only by a shrunken compressed residue. The destruction of the host cell now releases the gregarine either into the alimentary canal, where it lies between the epithelial lining and the peritrophic membrane, or into the body cavity of the larva where it may lodge itself in any situation to which the body cavity extends. The growth of the fly larva is accompanied by the growth of the gregarines until these have acquired their adult size. In a half grown larva gregarines at various stages may be seen since the oocysts continue to be eaten and develop as long as the larva continues in infected surroundings. Sections of a larva at this stage show many gregarines with a coelomic habitat but a still greater number occupying a position inside the alimentary canal, not among the food contents, but lying between the epithelial lining and the peritrophic membrane. This condition persists throughout the life of the sandfly larva.

If, now, a fly in the pupal or adult stage be examined, an entirely different picture is presented. The coelomic gregarines are present as before but those which had their habitat inside the alimentary canal have completely disappeared. In many hundreds of sections and dissections we have never seen a single gregarine within the alimentary canal in either the pupal or adult stages of the fly. In the larvæ, on the other hand, they are regularly seen, both in sections and dis-



Fig.7



Fig.8



Fig 9



Fig. 10

# Kahn Test Equipment for Syphilis

(as originally designed by Dr. R L. Kahn of Michigan, and used in Leprosy Research Laboratory, School of Tropical Medicine, Calcutta.)

Kahn test tubes	Rs	5 -	per gross
Vials, 2 drams, flat bottom	As	- 5	doz
Kahn pipette, 1 c c × 1 100th	Re	1 12	each
Ditto 2 10 c c × 1 1000th	Rs.	3 -	each
Ditto 5 c c	Re	1 -	each
Kahn Rack, copper, 30 holes in 3 rows	Rs	5 -	each
Test tube basket, small, 4" × 5" × 4"	Re.	1 4	each
Thermometer, 110 C	Rs.	2 8	each
Kahn Standard Antigen, 5 c.c	"	6 -	per phial
Ditto 25 c c	"	26 -	per phial

Or, in the alternative, the following —

Bacto Beef heart, dehydrated	Rs	3 -	per 25 grams
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A special illustrated pamphlet describing the method and technique of KAHN test will be gladly sent to CLINICAL workers, on receipt of annas four in stamps.

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#### EXPLANATION OF PLATE LIII

- Fig 11 Longitudinal section of abdomen of *P argentipes* showing 2/3 of the space occupied by oocysts of the gregarine Two gametocysts in different stages also present
- , 12 Two gametocysts, one indenting and compressing an adult gregarine
- .. 13 Two gametocysts, one in an early stage of division, the other with the number of gametes complete
- , 14 Two adult gregarines compressed against one another One unruptured gametocyst containing fully developed oocysts

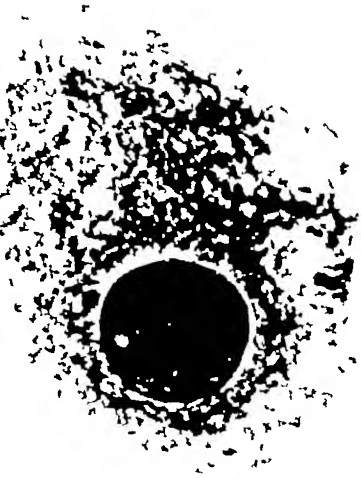


Fig 1



Fig 2



Fig 3



Fig 4

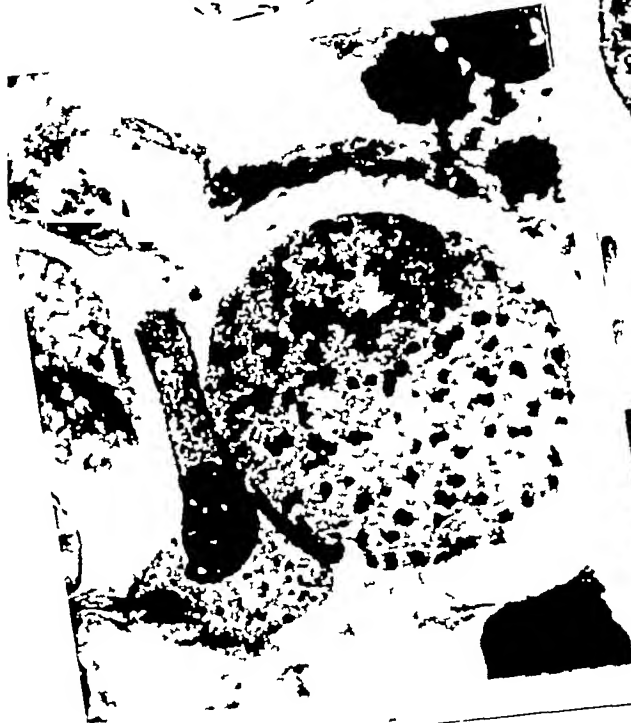


Fig 5



Fig 6







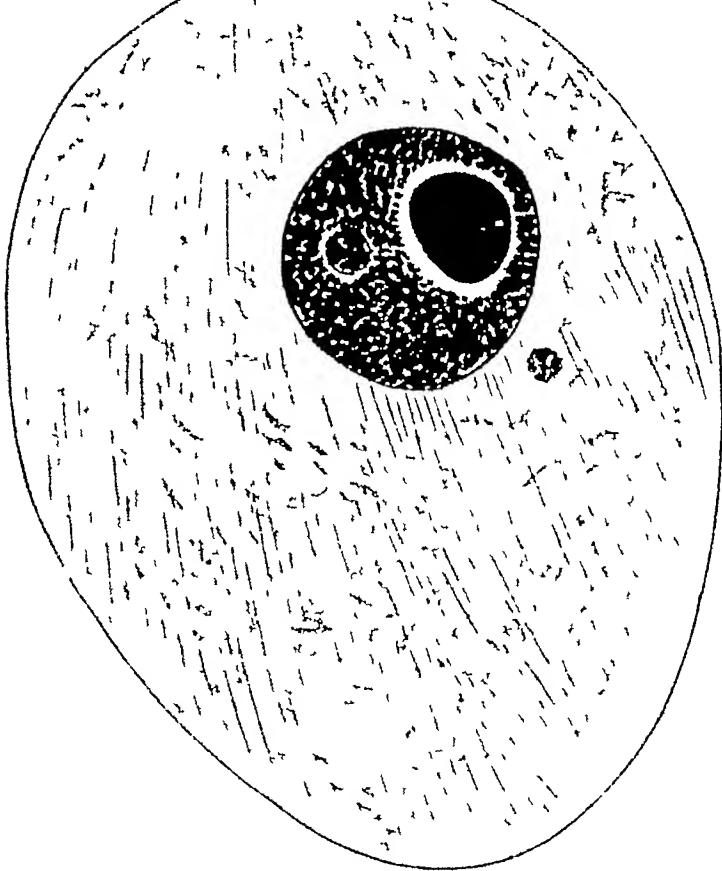
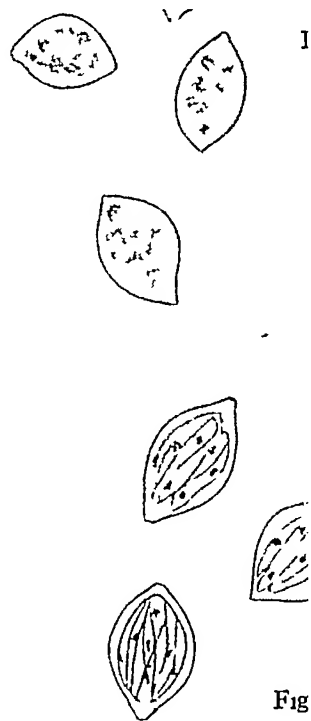


Fig.15



Fig



(b)



(c)

Fig.18



EXPLANATION OF PLATE LII

- Fig 7 Newly released sporozoites attacking the gut epithelium in larva of *P argentipes*
- „ 8 Young intracellular gregarine in epithelial cell of gut of larva of *P argentipes* Peritrophic membrane also shown
- „ 9 Longitudinal section of larva of *P argentipes* showing gregarines both within the gut and in the body cavity
- „ 10 Sagittal section of pupa of *P argentipes* showing heavy gregarine infection



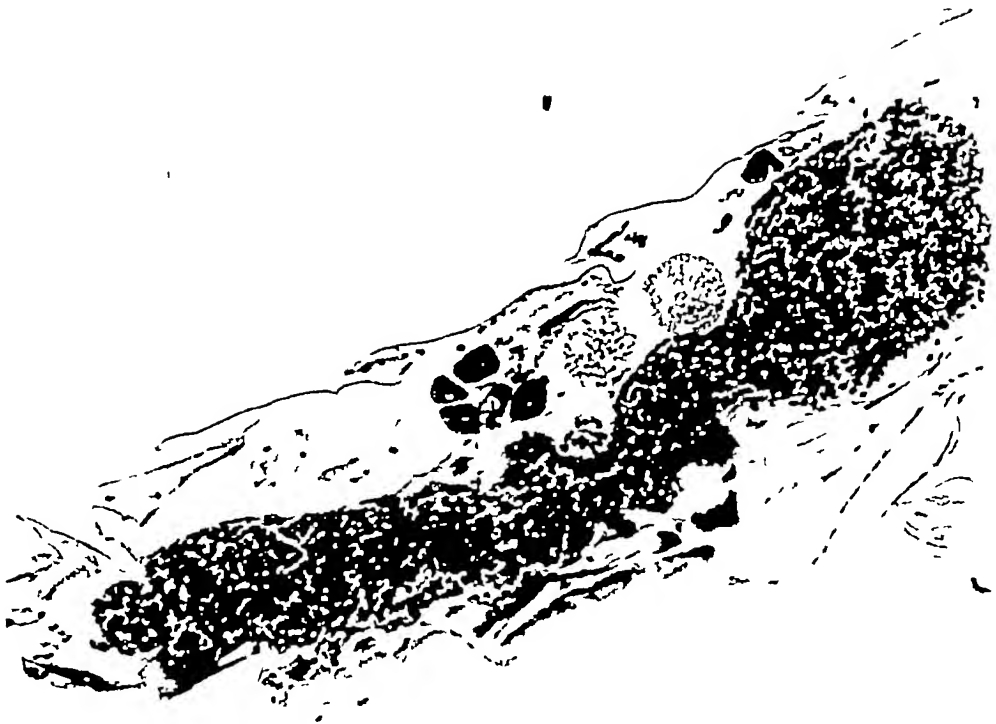


Fig.11



Fig.12



Fig.13



## FUNCTIONAL TESTS SELECTED

The liver performs many and varied functions, but not much is known definitely about all of them. Out of the functional tests devised to test some of the better-known functions, we have selected the following tests most frequently used in clinical laboratories —

- (1) Levulose tolerance test based on the relation of the liver to carbohydrate metabolism
- (2) The Van den Bergh reaction, depending on the secretion of bile by the liver
- (3) Nitrogen partition of blood, based on the relation of the liver to protein metabolism
- (4) Bromsulphalein dye test of Rosenthal and White (1925)

## METHOD OF INVESTIGATION

All of these four tests were carried out on each patient at one time, thereby making a complete study, and avoiding annoyance to the patient and the possible difference in the function if the tests were done on different days. The procedure suggested by Rubenstone and Tuft (1926) was adopted.

The patient was prepared as for glucose tolerance test, i.e., he was given no food after the evening meal. The test was always started between 8 to 9 in the morning while the patient had not broken the fast. The determined amount of levulose as indicated below was dissolved in about 150 c.c. of distilled water and flavoured with a little fresh lemon juice, and also the calculated amount of bromsulphalein dye (prepared by Hynson, Westcott and Dunning) was drawn into a syringe and kept ready. Then 10 c.c. of blood was drawn from a vein, 5 c.c. was oxalated using Folin's (1919) lithium oxalate cloth, and the remaining 5 c.c. were allowed to clot for the separation of serum. Immediately after the blood had been drawn the dye was injected into the same vein, and the patient given levulose solution to drink. Blood was then withdrawn 30 minutes, one hour, two hours, and three hours after the injection. The 30 minutes sample (about 5 c.c.) was drawn from a vein of the other arm, about 2 c.c. was oxalated and the rest allowed to clot. The remaining samples were withdrawn from the fingers by needle punctures, and oxalated. The blood from the finger was allowed to drop into a small porcelain crucible containing a particle or so of lithium oxalate.

The serum, collected before the dye was injected, was used for the Van den Bergh test, and also as the standard for the dye test. The oxalated blood collected before the injection was used for the determination of non-protein nitrogen, urea nitrogen and sugar. The sample of serum drawn 30 minutes after the injection of the dye was used to determine the retention of the dye as described by Rosenthal (1925). Blood sugars were done on all the other oxalated blood samples. 0.1 c.c. of the oxalated blood was taken up in an accurate 0.1 c.c. special pipette and laked immediately for either Folin or Hagedorn determination of sugar.





## Results

No	Name	Fasting blood sugar Mg	LEVULOSE TOLERANCE TEST					PARTITION OF BLOOD NITROGEN			VAN DEN BERGH REACTION		Brom Sulphate in Dye Per cent of dye retained in 30 mts	REMARKS
			1/2 hr after levu lose Mg	1 hr after Mg	2 hrs after Mg	3 hrs after Mg	Max rise Mg	Dura- tion of rise Hrs	N P N Mg	Urea N Mg	U N P N Per cent	Imme diate Direct Units	Indirect Units	
1	S K P	67	85		87		21	2?	35.7	13.5	38	0	0.4	0
2	W G	87	104	124		145	58	3+				0	0.8	10
3	R M	115	139	179	206	194	91	3+				0	1.0	0
	R M.(a)	126	126		129	133	7	3+				0		
4	C S	83	92	107	104	88	24	3	37.0	7.9(c)	21	0	0.4	0
5	S P	118		131	143	117	25	3+	39.2	11.6(c)	29	0	0.5	0
6	C Sa		101	138	126	103	27	3?	20.5	9.8	48	0	0.4	0
7	MoA	95	99	90	87	100	0	0	32.8	13.8	42	0	3.0	0
	MoA (b)											0	2.0	
8	Mrs N	96	83	89	102	88	6	2?	26.0	12.3	47	0	4.0	0
9	E W J W	129	115	127	123	117	0	0	30.1	12.6	41	0	1.1	5(d)
10	J D	74	80	88	85	71	14	2+	21.3	10.1	47	0	0.3	0
11	H C	98	101	110	113	103	15	2+	25.2	10.8	42	0	1.1	0
12	Mrs G	74	85	85	95	88	21	3+	30.9	11.4	37	0	0.4	0
13	N	106	115	115	124	115	18	3+	23.5	12.5	53	0	0.6	0

(a) This is a second levulose test done on R M on 2.2.27, the first one was done on 19.1.27, patient's condition was unaltered

(b) This is a second Van den Bergh test done on 10.6.27, patient in extremis, the first test was done on 18.2.27

(c) Urea determination done 26 hours after blood was drawn

(d) Less than 5 per cent, there was colour, but weaker than 5 per cent standard

## EXPLANATION OF PLATE LIV

- Fig 15 Adult gregarine showing striations on epicyte and one cytoplasmic inclusion
- „ 16 Oocysts as seen in fresh preparations
- „ 17 Oocysts stained with iron-haematoxylin
- „ 18 Stages of mitosis in nuclear division within the gametocysts
- (a) Resting nucleus with karyosome and nuclear membrane
  - (b) Nuclear membrane absent Karyosome disappeared and chromatin irregularly arranged in comparatively large masses
  - (c) Chromatin in thick rod-like masses (chromosomes)
  - (d) Splitting of chromosomes and formation of an equatorial band  
Nuclear spindle present and nucleus becoming elongated
  - (e) Separation of the split chromosomes to opposite poles of the spindle Nucleus much elongated
  - (f) Aggregation of chromatin in irregular masses and constriction of nuclear spindle
  - (g) Comet-like appearance of the two daughter nuclei after parting of the spindle at its centre
  - (h) Two daughter nuclei with nuclear membranes Ready to proceed at once to further division

In sprue the gastro-intestinal tract is affected and there is marked emaciation. The rise and retarded disappearance of levulose from the blood may be due to the same factors as are concerned in fasting animals in Mann's experiments. The repetition of the test in one case (R M) who had yielded a rise of 91 mgs lasting for three hours a fortnight previously, gave a normal curve. Obviously we cannot take levulose tolerance by itself a sufficient indication of liver inefficiency.

*Nitrogen Partition of Blood*—Normally urea nitrogen makes up from 40 to 50 per cent of the non-protein nitrogen content of the blood. Of our eleven cases in which nitrogen partition was studied, four show urea nitrogen below 40 per cent of the non-protein nitrogen and only two sufficiently below to be abnormal. And we are doubtful about the accuracy of both of these results, as the determinations were not done the same day the blood was drawn. There was the technical difficulty of distillation, and duplicates could not be done.

It is also to be noted that although Bollman, Mann and Magath (1924) found no formation of urea in the dehepatized animals they were not able to demonstrate any decrease in its formation even in those animals where reduction in the amount of hepatic tissue was greatest.

*The Van den Bergh Reaction*—Although the mechanism of Van den Bergh reaction is not completely understood, some factors in connection with it have been made clear by Mann (1925). It is definitely proved that bilirubin is derived from hæmoglobin, that though the liver does make bilirubin, more of it is made by the spleen and bone-marrow. The amount of bilirubin formed and the rate at which it forms depend on several factors, and one of these is the availability of hæmoglobin. The amount of hæmoglobin available for making bilirubin depends on factors such as destruction of blood, diet, and loss of blood.

Rosenthal in his work on dye excretion by liver has shown that bilirubin circulates firmly bound to the serum proteins. This prevents its elimination by the kidney. Hall (1927) assumes that in the bound condition bilirubin does not react with diazo reagent to give an immediate direct Van den Bergh reaction. The addition of alcohol to serum liberates bilirubin which now reacts with diazo reagent to give the indirect reaction of Van den Bergh. Bilirubin combined with serum proteins circulating in the blood-stream on reaching the liver is extracted by liver cells and excreted along with the bile salts into the bile canaliculi. Bile salts evidently liberate bilirubin from its combination with the serum proteins, and bilirubin in this state in the bile canaliculi, or after its absorption into blood-stream, if there is any obstruction to the outflow of bile, reacts directly with diazo reagent to constitute the immediate direct reaction of Van den Bergh test.

None of our cases gave an immediate direct reaction. The indirect reaction varied from 0.4 to 9.9 units. McNee gives the normal range of bilirubin from 0.2 to 0.6 units. Six of our 12 cases show no more than 0.6. The readings for the other six are 0.8, 1.0, 1.1, 1.1, 4.0, 9.9. The indirect reaction higher than the normal may mean the destruction of the red cells at a rate higher than the normal, or defective extraction of the bilirubin by the liver, or both. But we agree with Greene and Connor (1926) that the serum bilirubin in such cases

# LIVER FUNCTION IN SPRUE

BY

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THERE are scattered references in literature to the effect that the liver is affected in sprue. Brown (1908) was one of the earliest writers to say that the liver was reduced in size in sprue even to about half its normal size. Begg (1912) in his study of 100 cases, noted the reduction in the size of the liver as a most marked sign of active sprue. He did not find the liver to be cirrhotic, but normal and merely reduced in size. Even the reduction in size was evidently such that he found within 24 hours of administering the first dose of santonin the liver returned to the normal size, and the santonin in his opinion acted as a 'germicide' in the intestines. More recently Mikeladze (1926), in reporting some (10) cases of sprue in Georgia states that the diminution in the size of the liver is a constant feature of all cases of sprue unless sprue, is complicated by malaria. Wood (1925) on the contrary remarks 'the liver is vaguely described as atrophied, but there seems to be slight evidence that this atrophy is anything more than that shared by all the tissues

The present study was, therefore, undertaken to see if modern functional tests would throw any light on the efficiency of the liver in sprue.

Thirteen cases of well marked sprue have been studied. All the cases were such as could be clinically described as definite cases of sprue. Emaciation, passage of bulky, frothy and pale stools, specially in the morning, involvement of tongue, and anaemia were the diagnostic features.

## SUMMARY AND CONCLUSIONS

Thirteen cases of well marked sprue have been studied. Nitrogen partition does not reveal any inefficiency of the liver. The bromsulphalein dye retention test has given negative results, except in one case in which it may well have been due to the extreme lowering of vitality generally on account of the approaching dissolution—the test was done three days before the patient died. Increase in the serum bilirubin in six cases, as shown by the indirect Van den Bergh reaction, indicates increased destruction of erythrocytes rather than defective function of the liver. If the liver had been at fault, the dye test would have shown a parallel retention. Seven cases gave abnormal levulose tolerance curves, but on account of the non-specific nature of this test, and negative results yielded by the other tests, we cannot interpret our abnormal curves in terms of liver inefficiency. In sprue, inanition may more likely be responsible for the abnormal levulose tolerance curves than the condition of the liver. All the tests, except nitrogen partition, which was not done, give parallel results and point to liver inefficiency in one case (W. G.) only, and as we have said before he was *in extremis*.

Our investigation, therefore, shows that in sprue the liver is not affected to such an extent as to show impairment by liver function tests.

We wish to thank Lieut-Col F. P. Mackie, I.M.S., for his keen interest in this investigation and for his giving us the benefit of his large experience of sprue by confirming the clinical diagnosis of every case included in this study. Our thanks are also due to Dr N. Hamilton Fairley for suggesting this enquiry. We are deeply indebted to Major P. K. Gilroy and Dr Jivraj Mehta and their colleagues for giving us every facility to study sprue cases in their hospitals.

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The dosage of levulose used by Spence and Brett (1921) and Tallerman (1923) was —

To a person weighing	80	kilos	50	gms
Do	60	,	40	gms
Do	40	,	30	gms

For convenience of calculation we have made the dosage proportional to the weight of the patient, and given  $\frac{1}{2}$  gm of levulose per kilo body-weight. This circumstance will not detract from comparativity of our results with those of other investigators. The difference introduced is a slight one, and Tallerman asserts that his results with his 30 gm to 50 gm dose are strictly comparable with the results of other investigators who have used 100 gms as a routine dose. The dosage of bromsulphalein was calculated on a basis of 2 mgs per kilo of body-weight. We have used throughout a 5 per cent solution of the dye supplied in sterile capsules by Hynson, Westcott and Dunning. The dosage of this solution in cc was obtained by dividing the body-weight of the patient in pounds by 55.

#### METHODS OF ANALYSES

Blood non-protein nitrogen and urea nitrogen were determined by Folin's (1919) method.

Blood sugars were done either by Folin's new method or by Hagedorn's methods, the two methods agreed closely.

For Van den Bergh test we have used with slight modifications, the improved technique described by McNee and Keefer (1925). We did not use caffeine sodium salicylate, its effect was inconstant. For the sake of clearness we describe below the method used by us.

**Direct reaction**—Take 3 small test tubes (size 5 cm  $\times$  0.7 cm) and to each add 0.25 cc of serum to be tested. To tube 1 add 0.2 cc of water and to tube 3 add 0.2 cc diazo reagent made freshly. Wait for five minutes for the reaction to become complete in the control tube No. 3, then add 0.2 diazo reagent to tube 2. We have considered the reaction to be positive if the reaction began within 30 seconds of the addition of reagent to tube 2. We have included the so-called biphasic reaction, when the reaction began before the lapse of 30 seconds, among the positive results.

**Delayed reaction**, where the reaction did not begin until after 30 seconds, we have included in our negative results.

**Indirect reaction**—Take 1 cc serum to be tested in a 15 cc graduated centrifuge tube and add 0.5 cc freshly prepared diazo reagent. After two minutes add 2.5 cc 95 per cent alcohol. Stir with a rod and add 1.6 cc saturated ammonium sulphate solution. Stir with a rod. Centrifuge. All the resultant colour is carried into the supernatant alcoholic solution and little or none goes down with the precipitate. The quantity of supernatant fluid is read on the graduations of the centrifuge tube and the dilution of the bilirubin contained in one cc of serum is thus directly obtained. This varies from 2 to 3 cc. The supernatant alcoholic coloured solution is pipetted off from the centrifuge tube into the cup of a plunger type of colorimeter and compared against the standard solution.

## APPENDIX

### *Case Histories*

1 S K P, European male, aged 19 First attack, duration two months Stools whitish yellow, pultaceous with gas, one or two in the morning Tongue sore, ventral surface raw, history of ulcer frenum Weight fell from 9 st 2 lbs to 7 st 12 lbs R B C 4.3 millions, polychromatophilia, poikilocytosis, no nucleated cells Hb 75 per cent (Sahli) Calcium in blood 10.8 mgs per cent

2 W G, European male, aged 38 First attack, duration nine months Stools characteristic, two or three a day Tongue sore, history of ulcers Extreme emaciation, weight, 5 st 10 lbs representing a loss of 4 stones R B C 2.5 millions Anisocytosis, no nucleated cells Hb 65 per cent (Sahli) Calcium in blood serum 10.1 mgs per cent

3 R M, European male First attack, duration six months Stools (day of examination) deep brown, bulky, and pultaceous Tongue said to have been sore Loss of weight two stones R B C 3.3, millions, polychromatophilia, poikilocytosis, no nucleated cells Hb 75 per cent (Sahli) Calcium in blood serum 10.5 mgs per cent

4 C S, Anglo-Indian male, aged 48 Second attack, three months' duration First attack two years ago Stools five or six a day, fluid, yellow, but no gas Tongue not involved at time of examination, but gives history of soreness Emaciated R B C 4.1 millions, marked anisocytosis poikilocytosis and polychromasia Hb 88 per cent (Sahli) Calcium in blood serum 10.8 mgs per cent

5 S P, Anglo-Indian male, aged 40 First attack, duration one and a half months Stools, six or seven daily, light, soft and bulky Tongue, minute ulcers, dorsum, 6½ lbs loss of weight R B C 5.3 millions, blood picture normal Hb 70 (Sahli) Calcium in blood serum 10.3 mgs per cent

6 C S a Anglo-Indian male, aged 26 Fifth attack duration five months Four attacks during the previous five years of various duration Stools, four or five daily, yellowish white, soft, bulky, gas present Tongue sore and congested, history of ulcers Lost 32 lbs in weight during this attack, emaciated R B C 1.4 millions Changes in size and colour of cells, but no nucleated cells Hb 30 per cent (Sahli) Calcium in blood 10.4 mgs per cent

7 McA, European male, aged 32 First attack, duration fortnight, gives history of 'indigestion' for two years, no dysentery Stools, eight a day, pale bulky and frothy Tongue sore and congested Says has lost 2 stones during the last four years Not emaciated (When seen four months later, was extremely emaciated) R B C 1.7 millions, poikilocytosis, anisocytosis and

$\frac{\text{Standard}}{\text{Unknown}} \times \text{dilution of unknown} = \text{Units of bilirubin}$

The standard solution was made as suggested by McNee and Keefer (1925) by dissolving 2.161 gms anhydrous cobaltous sulphate in 100 c.c. water. The colour of this solution represents one unit or 0.5 mgs of bilirubin per litre of serum.

### DISCUSSION

*Levulose Tolerance Test*—McLean and de Wesselow (1921) did not find any marked rise in normals after ingestion of levulose. They give no figures, but from Chart V of their paper it appears that in the only case charted they did not get a rise higher than 8 mgs after 50 gms of levulose. Spence and Brett (1921) state 'The normal blood sugar curve after 50 gms of levulose can thus be represented by a continuous straight line from the original blood sugar level, or a line showing slight oscillatory deviation of 5 to 10 mgs per 100 c.c. from the original level'. King (1927) gives an average rise of 8 mgs from the fasting level in normal individuals—actual variation in his case was from 5.18 mgs. Tallerman (1923) found an average rise of about 15 mgs for his normals. Besides the actual value of increase in sugar level the duration of the rise is also a matter of importance. In the normal cases cited, the blood sugar returned to the fasting level within 1½ hours. Taking the highest figure (18 mgs) of King (1927), we may say that a rise of 18 mgs coupled with the non-return of the blood sugar level to the fasting level in 1½ hours is indicative of abnormality. Judged by these standards seven out of our thirteen cases of sprue show an abnormality by the levulose tolerance test.

But what is the validity of the test? Does an abnormal levulose sugar curve really show hepatic inefficiency, as distinct from inefficiency of the other tissues? Evidence in favour is that Mann (1925) has demonstrated that the liver has an important function in relation to carbohydrate metabolism, and is concerned with maintaining the blood sugar level which is a physiological constant. The other authors cited who have used levulose as a test of hepatic efficiency find that an abnormal sugar curve is obtained in diseased conditions in which the liver is definitely involved. Against this has to be considered the remarkable work of Mann and Bollman (1926) who found that when a glucose (or levulose) tolerance test is done on an animal which has been fasted for four or five days, the rate at which the blood sugar level returns to normal is very much retarded, much more so than in an animal 4/5th of whose liver has been removed, in the latter case retardation is only very slight, not lasting more than 60 minutes. They also found that the intravenous injection of levulose into dehepatised dogs did not raise the blood sugar level, and came to the conclusion that the body can utilize levulose without the liver.

Though the liver is a vital factor in carbohydrate metabolism, there are other tissues equally concerned, for example, the pancreas and muscle. The abnormal levulose curves obtained by various authors, in conditions in which the liver was involved, may be due as much to the liver as to other tissues which may be involved at the same time. The observation that the body can utilize levulose without the liver leaves us no other option.





is an index to the severity of the hæmolytic process rather than to the disturbance in the liver.

Fairley and Mackie (1926) in their series of 16 cases, noted indirect reaction higher than normal only in seven cases, and these figures when compared with figures for pernicious anæmia given by Andrews (1924) were generally lower. They suggested that the indirect Van den Beigh reaction might be used for differentiating sprue from pernicious anæmia. We do not think this reaction can help us to distinguish the two conditions. In the first place, two of our cases and one of theirs gave figures as high as pernicious anæmia cases of Andrews—20, 44, 30. Secondly, notice has to be taken of the dynamic nature of the processes involved. The indirect reaction is merely a measure of the amount of bilirubin in combination in the blood-stream, this quantity, if the liver be functioning normally, depends on the amount of the red cells available and the rate at which they are being broken up. It is evident that in the course of any given case of pernicious anæmia, both these factors will vary within very wide limits and so will the indirect reaction. Brown, Ames, Warren and Peabody (1925) who followed, in case of pernicious anæmia, the changes in the plasma bilirubin over a period of time, report the close relationship between an increase in this pigment and the clinical exacerbations of the disease. Further Andrews' (1924) figures do not give a comprehensive picture of the bilirubin changes in pernicious anæmia. Greene and Conner (1926) reporting twelve cases of this disease say 'In the majority (eleven) the amount of serum bilirubin was at the upper limit of the normal or slightly increased beyond these limits'. We can say exactly the same of our cases of sprue. Hæmolysis is a feature of the disease, evidenced by the low blood counts (*see* appendix), the intensity of the process varies from time to time and is reflected in the indirect reaction readings. One of our cases (McA) whom we examined at an early acute stage gave an indirect reaction of 30 units, four months later the indirect reaction of the same patient was 20 units, though the general condition of the patient was very much worse.

*The Dye Test*—In the bromsulphalein dye test of Rosenthal and White (1925) we have a liver function test of a type different from the other three tests used by us. Extraction of the dye from the blood-stream depends on the liver alone, while in the other tests the liver was only one of the tissues concerned. But it must be noted that though the test is specific for the liver, it is not clear yet whether it yields information on all the different functions of the liver.

Mann and Bollman (1926) found the retention of the dye most marked in those dogs that had lost the greatest amount of hepatic tissue. But even in this case the retention was not proportional to the loss of tissue. The dye was retained indefinitely if the dog was completely dehepatized, but appreciable retention did not manifest itself until a considerable amount (about 4/5) of the liver had been removed.

Out of our thirteen cases, dye retention was observed in two cases only 10 per cent in one, and less than 5 per cent in the other. 10 per cent dye retention was obtained in a patient (W. G.) *in extremis*, he died three days after the test was done. Less than 5 per cent retention is too small to be significant.

The peripheral blood of the patients, who were diagnosed as suffering from kala-azar by the clinical symptoms, aldehyde and antimony tests, was carefully examined for the presence of Leishman-Donovan bodies. In those cases in which no organisms could be detected after a careful and prolonged search, a therapeutic dose of urea-stibamine (0.05 to 0.1 gm) or one of the other antimony compounds was given intravenously and the blood was examined 10 minutes, 20 minutes and 30 minutes after the injection. Table A shows the results obtained in 13 out of a series of 26 cases examined in this way.

A perusal of the table will show that the slides of blood taken 10 minutes after the injection showed Leishman-Donovan bodies in 8 out of 13 cases, the slides taken 20 minutes after also showed the presence of the organisms in 8 out of 13 cases and those taken 30 minutes after showed them only in 2 out of 13 cases. It can be concluded, therefore, that the injection of antimony compounds has the effect of liberating these bodies from the endothelial cells and expelling them into the general circulation. It would further appear that the action of antimony compounds in this respect is not very prolonged. The highest number of parasites was found ten minutes after injection, they decreased in number in slides taken 20 minutes after, while they were present only in 2 slides taken 30 minutes after.

In Table B we have given results of a similar examination carried out on the remaining 14 cases of the series.

A perusal of the table will show that most of these patients had clinical evidence of kala-azar and the aldehyde as well as the antimony tests were positive. No Leishman-Donovan bodies were found in the peripheral blood and even after provocative doses of antimony were given, the blood was negative after a prolonged and careful search. In these cases we further searched for the presence of the parasites by means of spleen puncture, where that was possible, or by means of blood culture examination where spleen puncture could not be done. In the 'remarks column' of the table we have given the results of this examination. It will be seen that in 7 out of 14 cases examined we could not detect the presence of Leishman-Donovan bodies by these methods. The remaining cases were from the out-patient department and in these, further examinations by spleen puncture or cultural method, unfortunately, could not be done.

## DISCUSSION

From the above it would appear that intravenous injections of compounds of antimony in man, produce effects similar to those seen in lower animals, viz., engorgement and increase of rhythmic movements of liver and spleen. Such phenomena would result in rupture of the endothelial cells and the liberation of the Leishman-Donovan bodies, contained therein, into the general circulation. The extent to which this occurs will naturally depend on the condition of these organs. In those patients in whom the liver and spleen are much enlarged, firm and inelastic owing to marked fibrotic changes in the reticulo-endothelial tissues, neither any marked degree of dilatation nor much increase in their rhythmic movements would be possible under these conditions.

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TABLE B

Case No	Duration	Spleen	Aldehyde	Antimony Test	<i>Examination of Peripheral blood for Leishmania donovani</i>				REMARKS
					Before giving injection	10 minutes after	20 minutes after	1½ hours after	
1	7 months	Inches 2	±	+	Nil	Nil			No L-D body on spleen puncture
2	1½ months	1½	±	+	Nil	Nil			Probably early kala azar
3	4 months	2	±	+	Nil	Nil			Blood culture negative
4	3 years	3	±	+	Nil	Nil			Blood culture negative
5	1 year	2	+	+	Nil	Nil			Partially treated outside
6	3 years	? Ascites	++	+	Nil				No L D body on spleen puncture
7	3 years	8	+++	+	Nil	Nil			Relapsed, discharged only one week before from Carmichael Hospital
8	1 month	3	+	+	Nil	Nil			No L-D body on spleen puncture
9	1 year	4½	—	+	Nil	Nil			No L-D body on spleen puncture
10	1 year	6	+++	+	Nil	Nil			No L-D body on spleen puncture
11	7 months	3	+++	+	Nil	Nil			Out patient case
12	1 year	4	+++	+	Nil	Nil			Do
13	10 months	6	+++	+	Nil	Nil			Do
14	10 months	8	++	+	Nil	Nil			Do
									No L-D body on spleen puncture

Aldehyde test + = positive, — = negative, ± = doubtful, ++ strongly positive, +++ very strongly positive  
Antimony test + = positive

achromia, but no nucleated cells Hb 40 per cent (Sahli) Calcium in blood serum 10.4 mgs per cent

8 Mrs N, Anglo-Indian female, aged 47 Second attack of one month's duration Previous attack eight years ago Stools three or four in the morning, yellowish, formed, not frothy Tongue congested up and sides Emaciated RBC 4.4 millions Very slight poikilocytosis Hb 70 per cent (Sahli) Calcium in blood serum 10.1 mgs per cent History of dysentery four years ago

9 E W J W, Anglo-Indian male, aged 55 Second attack two months' duration Previous attack four years ago Stools six to eight daily, generally in the evening, pale yellow, bulky, soft but formed Tongue sore, congested patches, buccal mucous membrane Emaciated RBC 3.8 millions, poikilocytosis Hb 65 per cent (Sahli) Serum calcium 10.5 mgs per cent

10 J D, Indian male, aged 37 Third attack, duration one month, 1st attack in 1924, 2nd attack in 1926, 1st and 2nd attacks characterised by diarrhoea and sore tongue Present attack tongue is pale but not sore Stools one or two, yellowish, formed, frothy Lost 20 lbs in three years RBC 1.2 millions, anisocytosis, poikilocytosis, and achromia Hb 37.5 (Sahli) Serum calcium 9.9 mgs per cent History of dysentery a year ago

11 H C, Anglo-Indian male, aged 51 Second attack, duration six months Previous attack three years ago Stools three in the morning, whitish yellow, bulky and frothy Tongue congested and sore, blisters on tip Emaciation Loss of 3 st 6 lbs during the present attack RBC 2.4 millions, anisocytosis, and slight poikilocytosis, no nucleated cells Hb 55 per cent (Sahli) Serum calcium 9.8 mgs per cent

12 Mrs G, Anglo-Indian female, aged 59 First attack, duration two months Stools three or four in the morning, pale, bulky and frothy Tongue, sore, congested and coated Patches on lips Emaciated RBC 2.8 millions, anisocytosis No nucleated cells Hb 60 per cent (Sahli) Serum calcium 12.2 mgs per cent

13 N, Indian male, aged 30 First attack, says has lasted off and on for about a year Stools two or three a day, pale, bulky, frothy, but formed Tongue and buccal mucous membrane congested, patches and ulcers RBC 3.2 millions anisocytosis, poikilocytosis No nucleated cells Hb 55 per cent (Sahli) Serum calcium 9.9 mgs per cent

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# PROVOCATIVE ACTION OF ORGANIC COMPOUNDS OF ANTIMONY IN LEISHMANIASIS

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WHEN investigating the pharmacological action of organic compounds of antimony, the senior author noticed that intravenous injections of these compounds in animals produced a well marked increase in the volume of such organs as the spleen and the liver. This increase, although definitely present with antimonyl tartrates, was not very marked, but small doses of organic compounds of antimony such as urea-stibamine or Von Heyden's compounds produced a remarkable and persistent expansion of the spleen volume. Not only does this organ increase in size but its rhythmic movements are also strongly stimulated. That a similar state of affairs is brought about in man after intravenous injections of antimony compounds in therapeutic doses, is shown by the fact that most of the patients complain of a feeling of fullness and discomfort in the region of the liver and spleen, especially the latter. It seems probable that the dilatation of these organs is a part of the curative action of these compounds in kala-azar. It is well known that in this disease the spleen and the liver become the habitat of *Leishmania donovani*. Influx of large quantities of blood charged with antimony into these organs, probably facilitates the destruction of pathogenic protozoa present therein. The Leishman-Donovan bodies lie inside the endothelial cells present in various organs, engorgement and increase in the rhythmic movements of which would probably rupture these cells, in this way liberating the parasites which find their way into the peripheral circulation.

At the suggestion of Lieut-Col H W Acton, I M S,\* we carried out a series of observations to see whether this actually happens in clinical practice.

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\* After the paper on 'An Experimental Investigation into the Organic Compounds of Antimony' was published by the senior author, Dr Napier informs me that he tried the provocative action of antimony compounds on a few kala-azar cases. His results, however, were not published.—R N C.



excised uterus of cats or rabbits was always inhibitory, the action on the guinea-pig's uterus being less constant. Acton (1921) summarised the action of quinine on the pregnant uterus as follows — Weak concentration as 1 in 300,000 produces no effect, concentrations of 1 in 150,000 produce contractions under certain conditions, while a concentration of 1 in 44,000 produces a tonic spasm which if sustained would cause asphyxia of the foetus. But this concentration would also cause severe toxic symptoms. Concentrations of 150,000 such as occur with large doses in the body, increase the strength of the intermittent uterine contraction and in the presence of a predisposition or an exciting cause the pressure produced by the increased contractions might be sufficient to cause rupture of the membranes or dilatation of the os and so bring on labour. Seggelke (1921) found quinine satisfactory for therapeutic abortion by giving 10 c.c. of a 5 per cent quinine hydrochloride solution by vein plus the same dose intramuscularly. This was of use only if contractions had started which were then re-inforced by the drug. At the same time cinchonism was fairly severe. Acton (1922) showed that on the excised guinea-pig's uterus quinine, quinidine, cinchonine and cinchonidine acted as follows — (a) On virgin uterus. In weak solution (1 in 75,000) all these alkaloids increase the amplitude of uterine contractions. In stronger concentrations (1 in 20,000) they depress both the amplitude and rhythm, the cinchonine series is more powerful than the cinchonidine series and the hydro alkaloids are more powerful than the natural alkaloids. (b) On the multiparous uterus the behaviour is the same but the organ is more sensitive. (c) On the pregnant uterus, the previous observations by the same author were confirmed. Meyer and Gottlieb (1926) in their text-books of experimental pharmacology state that while quinine, if greatly diluted, produces but slight increase in tonus, large doses produce a relaxation. Nelson and Thomas (1927) state 'Quinidine, like quinine, stimulates the uterus to increased rate and amplitude of contractions in the pregnant as in the non-pregnant state. The stimulating action occurs through a relatively narrow range of concentration so far as the isolated uterus is concerned and the toxic depressor effect is very readily obtained. A marked or persistent increase in tonus was rarely seen in isolated uterus, and not at all in situ. In isolated uterus quinidine stimulates in smaller doses than quinine. In the intact animal very little difference was noted. No experimental data have been obtained to show that quinidine might be more effective as an oxytocic than quinine.' Two of us (R. N. C. and J. C. D.) (1927) found that quinamine, a dextro-rotatory alkaloid of cinchona bark, is very powerful in its action on the uterus of the cat and guinea-pig, dilutions as high as 1 in 500,000 producing moderate contraction and 1 in 200,000 a well-marked tonic contraction.

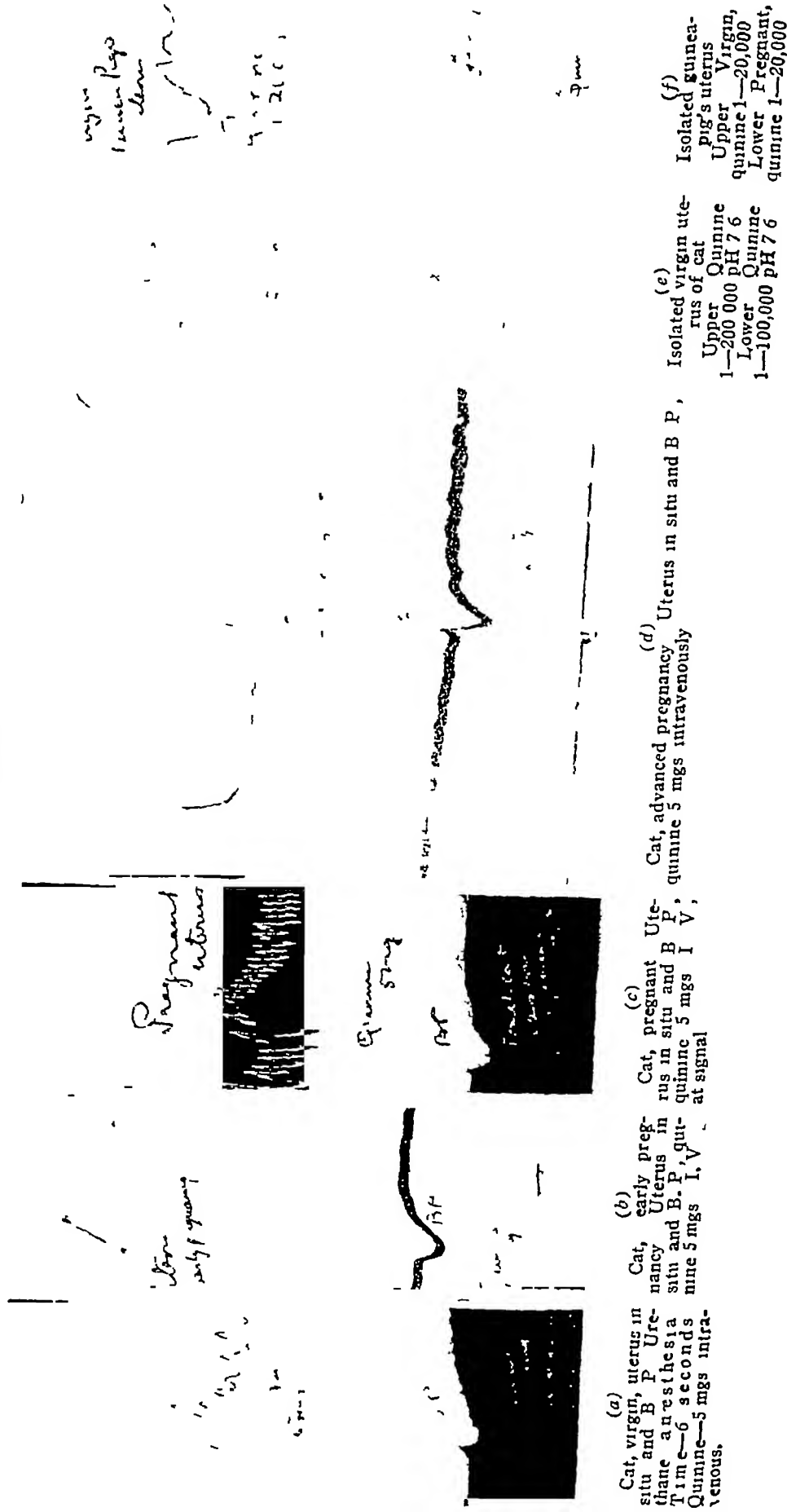
It will be thus seen that a great deal of confusion exists regarding the action of cinchona alkaloids and their derivatives on the uterus, we therefore carried out a large series of experiments to study the comparative action of these compounds on isolated and intact uterus. We not only studied the action of the principal alkaloids such as quinine, quinidine, cinchonine and cinchonidine but we also investigated the action of quinamine as well as some of the newer derivatives such as ethyl-hydrocupreine or optochin, ethyl-hydro-cupreidine, iso-heptyl-hydrocupreine, iso-heptyl-hydrocupreidine and iso-octyl-hydrocupreine or vuzin. The

TABLE A

Case No	Probable duration	Spleen	Aldehyde	Antimony Test	Examination of Peripheral blood for Leishmania donovani.					REMARKS
					Before giving injection	10 minutes after	20 minutes after	1 hour after	2 hours after	
1	3 months	Inches 3½	++	+	Nil	1 in 1	Nil	Nil		Relapsed case
2	6 months	3	++	+	Nil	Nil	1 in 1	Nil		
3	5 months	3	+++	+	Nil	2 in 1	1 in 1	Nil		
4	8 months	6	+++	+	Nil	Nil	1 in 1	Nil		
5	6 months	3	+++	+	Nil	1 in 1	Nil	Nil		
6	7 months	3	+++	+	Nil	Nil	1 in 1	Nil		
7	2 months	3	++	+	Nil	Nil	Nil	1 in 1		
8	1 year	8	+++	+	Nil	1 in 1	1 in 1	Nil		Had kala azar 2 years before
9	1 year	4	+++	+	Nil	1 in 1	Nil	Nil		
10	9 months	4	+++	+	Nil	5 in 1	3 in 1	2 in 1		
11	6 months	8	+++	+	Nil	1 in 1	Nil	Nil		
12	1 year		+++	+	Nil	Nil	1 in 1	Nil		
13	3½ months	3½	++	+	Nil	1 in 1	1 in 1	Nil		Relapsed case

Aldehyde test + = positive, ++ = strongly positive, +++ = very strongly positive. Antimony test + = positive

GRAPH I



The cases in Table B showed no parasites after injections of antimony compounds and we could not detect any parasites by spleen puncture or cultural methods. These findings are interesting as our clinical experience shows that there is a fair proportion of cases who give positive aldehyde and antimony tests, who show a leucocyte count of under 4,000 per cubic mm and who appear clinically to be suffering from kala-azar and yet by none of the methods at our disposal can we detect the presence of Leishman-Donovan bodies. This is especially so in cases in which kala-azar infection co-exists with malarial infection. These cases generally react very well to antimony injections, and where a slight elevation of temperature still persists after the course, a few doses of quinine bring the temperature down to normal. Why Leishman-Donovan bodies are not found in the peripheral blood or even in the liver and spleen it is difficult to say. Is it possible that in some cases the parasites are confined to such organs as the bone-marrow or other tissues and are difficult to get at? or is it possible that the presence of the malarial protozoon is inimical to *Leishmania donovani*? The two parasites have however been seen to exist together in the same organs. Those experienced in kala-azar work know that it is not always possible to get Leishman-Donovan bodies by puncture or cultural methods in cases which clinically are kala-azar and which also give positive aldehyde and antimony tests. Sometimes repeated punctures of spleen or liver have to be done or repeated cultures of peripheral blood have to be taken before a positive result is obtained.

In all the slides taken after injections of antimony compounds a well marked leucocytosis was noticeable. This, in our opinion, is due to the engorgement and increased rhythmic movements of the spleen and liver.

We tried to make a quantitative estimation of the parasites present in patients whose blood was positive to *Leishmania donovani* to see what effect the injections of antimony compounds produce on the number of these organisms circulating in the peripheral blood. The results, however, were so variable that no useful information could be gained.

## SUMMARY AND CONCLUSIONS

(1) Animal experiments show that injections of antimony compounds produce a well marked increase in the volume of such organs as the liver and the spleen and that there is also a remarkable increase in the rhythmic movements of these organs. That a similar state of affairs is brought about in man, after intravenous injections of these compounds, is shown by the fact that most of the patients complain of a feeling of fullness and discomfort after the injection.

(2) This is further borne out by the fact that in many cases the engorgement and increased rhythmic movements produce rupture of the endothelial cells and liberation of Leishman-Donovan bodies into the general circulation. The organisms, therefore, appear in the blood of these patients where they could not be detected before.

(3) Injections of antimony compounds, therefore, have a provocative action in those cases of kala-azar in which the fibrotic changes in the liver and the spleen are not too advanced.

We have noticed that when the tone is definitely raised the amplitude of the contractions has a tendency towards inhibition irrespective of the concentrations of the alkaloids used

#### *Cinchonidine*

(See Graph II)

*On the cat's uterus*—Cinchonidine has little or no action on the virgin uterus in situ, rarely it shows a slight increase in the amplitude of contractions after doses of 5 mgs intravenously. The multiparous non-pregnant, as well as the pregnant uterus respond in a similar manner.

The tone of the isolated virgin uterus is stimulated by such dilutions as 1 in 100,000 at pH of 7.2, but if the hydrogen-ion concentration is changed to 7.6, 1 in 200,000 dilutions also cause similar stimulation and a tonic contraction is produced (Graph II a). Higher concentrations like 1 in 50,000 and more also produce a moderate increase in tone.

The excised multiparous uterus behaves similarly (Graph II b).

*On the guinea-pig's uterus*—The isolated virgin uterus shows a slight increase in the amplitude of contractions in dilutions of 1 in 150,000 (Graph II b), 1 in 100,000 and 1 in 75,000 dilutions produce increase in the tone of the organ which becomes more marked when the strength of the solution is further increased (Graph II c). Weaker dilutions such as 1 in 200,000 do not produce any effect.

#### *Cinchonine*

(See Graphs II and III)

*On the cat's uterus*—5 mgs of cinchonine given intravenously produce a marked tonic contraction of the virgin uterus in situ (Graph II d). Increase in tone with acceleration of rhythm are produced in the case of the non-pregnant multiparous uterus (Graph II e). The pregnant uterus responds with a well-marked tonic contraction and increased rhythm (Graph II f).

On the isolated virgin uterus, dilutions of 1 in 150,000 produce an increase in the amplitude of the rhythmic contractions while 1 in 100,000 to 1 in 50,000 solutions produce a definite tonic contraction of this organ (Graph III a). The pregnant uterus responds with increased amplitude of contractions to dilutions of 1 in 100,000 (Graph III b), stronger concentrations from 1 in 50,000 to 1 in 20,000 produce a marked increase of tone with acceleration of the rhythm (Graph III c).

*On the guinea-pig's uterus*—The isolated virgin uterus does not respond to high dilutions such as 1 in 200,000 but concentrations of 1 in 150,000 to 1 in 20,000 or more produce a marked and lasting tonic contraction (Graph III d, e and f). The pregnant uterus is much more sensitive in this respect (Graph III g).

#### *Quinidine*

(See Graph IV)

*On the cat's uterus*—Quinidine does not produce quite consistent results on the intact uterus of these animals when administered intravenously in doses

# A COMPARATIVE STUDY OF THE ACTION OF CINCHONA ALKALOIDS ON THE UTERUS

BY

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THE pharmacological action of the derivatives of cinchona bark has been the subject of study by various investigators, but a perusal of the literature shows surprising variations in the findings based on experimental data. Kurdinowski (1906) stated that quinine strongly stimulated the contractions of the excised uterus. Zanda (1910) obtained relaxation of the excised uterus with quinidine though cinchonine and cinchonidine acted similarly to quinine. Worth Hall (1914) found that cinchonine, cinchonidine and quinidine induced strong contractions of the uterus with an increase in strength and regularity of the spontaneous movements and that the seat of action was directly on the muscle. Quinine was considerably weaker than the other three alkaloids, quinidine sulphate was the most active and appeared to be ten times as effective as quinine. These results were further confirmed by the same author (1916) by a series of experiments on the intact animals. Biberfeldt (1916), on the other hand, obtained only relaxation of the uterus with quinine in concentrations of 1—5,000 to 1—10,000 but this according to Sollman was probably due to some technical error. Smith and Fantus (1916) stated that the action of ethyl-hydrocupreine or optochin on the uterus, in the intact animal as well as on the isolated organ, was essentially like that of quinine. Bylsma (1920) quoted by Sollman, found that the action of iso-octyl-hydrocupreine or vuzine, iso-amyl-hydrocupreine and quinine on the

Hand-drawn geological map of the area around the 'Vulcan' mine. The map shows a central area labeled 'Vulcan' with a 'Vulcan Mine' and a 'Vulcan Shaft'. To the left is the 'Vulcan River' and to the right is the 'Vulcan Creek'. The map includes various geological features like 'Vulcan Hill', 'Vulcan Ridge', and 'Vulcan Valley'. A scale bar at the bottom indicates distances in miles (0 to 10).

(b)  
Isolated pregnant  
cat's uterus, cinchonine  
1 in 100,000 at arrow  
mark

(c)  
Isolated pregnant  
cat's uterus cinchonine  
1 in 20,000 at arrow  
mark

(d)  
Isolated virgin  
pig's uterus  
150,000 of cin

(c)  
Isolated virgin guinea-  
pig's uterus 1 in  
50,000 of cinchonine

(f) Isolated virgin guinea-pig's uterus 1 in 20,000 of cinchona





with higher concentrations the effect is proportionately increased (Graph V *f*) The isolated pregnant uterus responds to dilutions of 1 in 500,000 with a tonic contraction and increase of rhythmic movements The effects are more marked with higher strengths (Graph V *g*)

*Ethyl-hydrocupreine or optochin*  
(See Graph VI)

*On the cat's uterus*—On the virgin uterus in situ, intravenous injections of 5 mgs produce a tonic contraction with diminution of the amplitude of automatic contractions (Graph VI *a*) On the multiparous non-pregnant uterus the action is not so marked, there being usually a big contraction which partly relaxes leaving the organ in a state of increased tonus (Graph VI *b*) On the pregnant uterus optochin appears to have little or no effect (Graph VI *c*)

On the isolated organ dilutions of 1 in 200,000 slightly increase the tone, while 1 in 100,000 and more, produce a well-marked tonic contraction (Graph VI *d*, *e* and *f*)

*On the guinea-pig's uterus*—On the isolated organ, the action is essentially the same as on the cat's uterus (Graph VI *g*)

*Ethyl-hydrocupreidine*  
(See Graph VII)

*On the cat's uterus*—Doses of 5 mgs given intravenously produce an increase of amplitude of the rhythmic movements of the uterus in situ (Graph VII *a*) In the case of the pregnant and non-pregnant multiparous uterus, this effect is more marked and often a tonic contraction is produced, with acceleration in the rhythm of contractions (Graph VII *b* and *c*)

On the isolated organ, dilutions from 1 in 200,000 to 1 in 20,000 hardly produce any noticeable effect (Graph VII *d*)

*On the guinea-pig's uterus*—The virgin uterus responds with a well-marked tonic contraction with dilutions from 1 in 200,000 to 1 in 100,000 and less, the effect varies directly with the strength of the solutions (Graph VII *e* and *f*)

*Iso-heptyl hydrocupreine*  
(See Graph VIII)

*On the cat's uterus*—Doses of 5 to 10 mgs given intravenously produce little or no effect on the uterus in situ (Graph VIII *a*), rarely there is a very slight increase in tone On the excised organ dilutions of 1 in 100,000 to 1 in 20,000 have little or no effect (Graph VIII *b*)

*On the guinea-pig's uterus*—Concentrations from 1 in 200,000 to 1 in 20,000 produce little or no effect on the isolated guinea-pig's uterus, with strong solutions sometimes a diminution of the amplitude of contraction is noticed (Graph VIII *c*)

*Iso-heptyl hydrocupreidine*  
(See Graph VIII)

*On the cat's uterus*—The effect of iso-heptyl hydrocupreidine is stronger than that of iso-heptyl hydrocupreine Intravenous injections of 5 mgs cause a

four alkaloids quinine, quinidine, cinchonine and cinchonidine were manufactured by Merck's and were further tested and purified by Dr S' Ghosh, the Professor of Chemistry and his Assistant M<sub>1</sub> N R Chatterjee, and quinamine was kindly supplied to us by Mr G E Shaw, Quinologist to the Government of Bengal. Some of the derivatives of the cupreine and cupreidine series were prepared by Messrs Howard and Sons, while others were synthesised by the chemists of the Cinchona Enquiry. We are very grateful to Lt-Col H W Acton, I M S, for giving us sufficient quantities of these compounds for our tests.

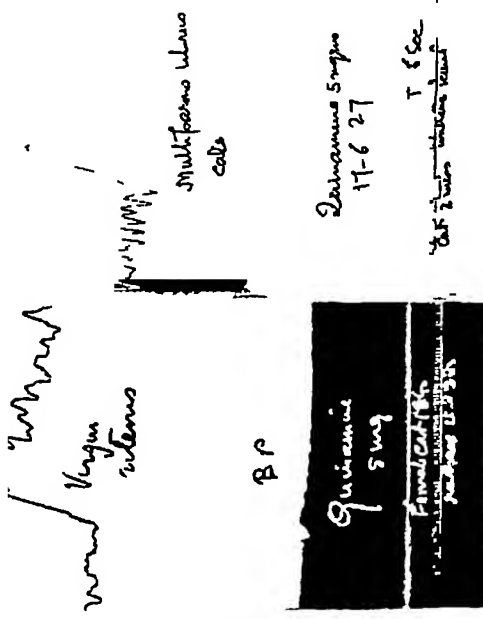
Our experiments were done on the uteri of cats and guinea-pigs. The action in situ was studied by a modification of Barbour's method described by Chopra and David (1927). The isolated uterus was studied by the technique described by Dale and Laidlaw, Fleish's solution being used for perfusion. The hydrogen-ion concentration of the solution was usually kept at 7.2 but we also did a number of experiments at 7.6. We noticed that while in the case of the isolated cat's uterus the organ worked most effectively in a solution with pH of 7.6, the guinea-pig's uterus worked best at 7.2. It was also observed that the guinea-pig's uterus was more sensitive and responded more readily to weak concentrations than the isolated uterus of the cat.

(See Graph I)

*On the cat's uterus*—The virgin uterus in situ shows slightly increased amplitude of the automatic contractions with 5 mg doses given intravenously (Graph I a), the multiparous uterus behaves similarly. The pregnant uterus shows well-marked tonic contraction with 5 to 10 mg doses, especially when the pregnancy is advanced, at the same time exhibiting increased rhythmicity (Graph I c and d). In early pregnancy there is often no definite increase in tonus but the amplitude of individual contractions is markedly improved (Graph I b).

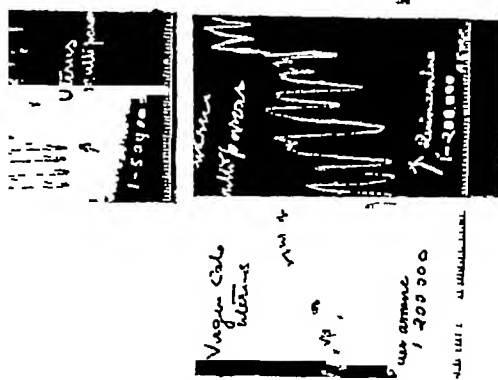
On the isolated cat's uterus, solutions of 1 in 200,000 to 1 in 100,000 at pH of 7.6 produce increase of tone in both the virgin and the multiparous non-pregnant uterus (Graph I e), there is, however, decrease in the amplitude of the contractions with acceleration of their rhythm. There is no response to dilutions such as 1 in 200,000 when the pH of the perfusing solution is 7.2. Stronger concentrations such as 1 in 50,000 and more, produce a well-marked increase in the tone of the organ. The isolated pregnant uterus (early pregnancy) is not markedly affected by concentrations of 1 in 100,000 or more, these merely produce a slight increase in the amplitude and rhythm without definitely increasing the tone of the organ.

*On the isolated guinea-pig's uterus*—In concentrations of 1 in 20,000 the tone of the virgin uterus is increased (Graph I f), with lower concentrations the rhythm is usually accelerated without any definite increase in tone. The multiparous uterus behaves in exactly the same way. A pregnant uterus usually gives no response to dilutions of 1 in 200,000 while dilutions of 1 in 50,000 to 1 in 100,000 merely produce acceleration of the rhythm, stronger concentrations such as 1 in 20,000 produce a well-marked increase in the tone and the amplitude of the contractions (Graph I f).

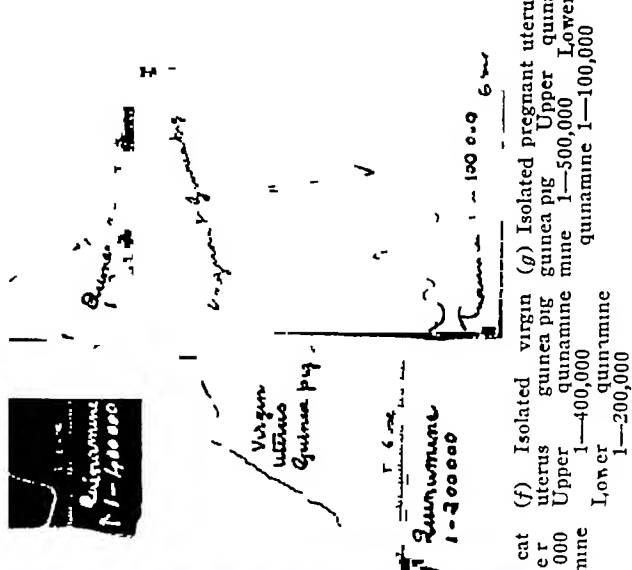


(a) Cat virgin uterus in situ and BP 5 mgs of quina mine I V, Urethane. Time-6 seconds

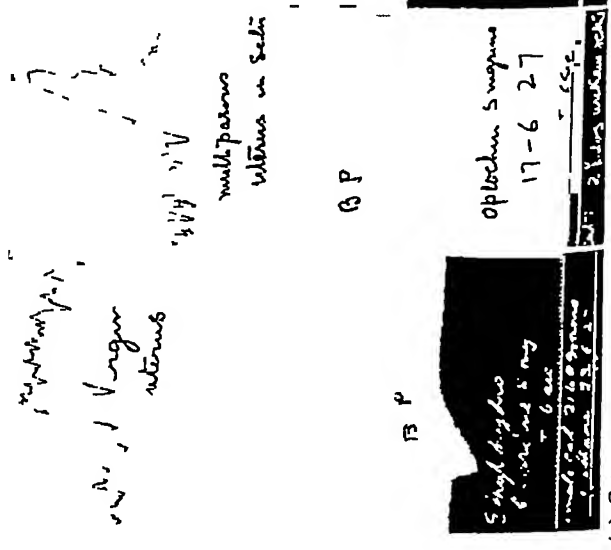
GRAPH VI



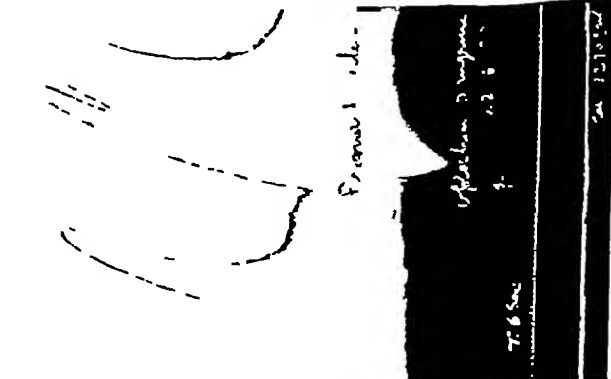
(b) Cat multiparous, uterus in situ and BP 5 mgs of quina mine I V, Urethane. Time-6 seconds



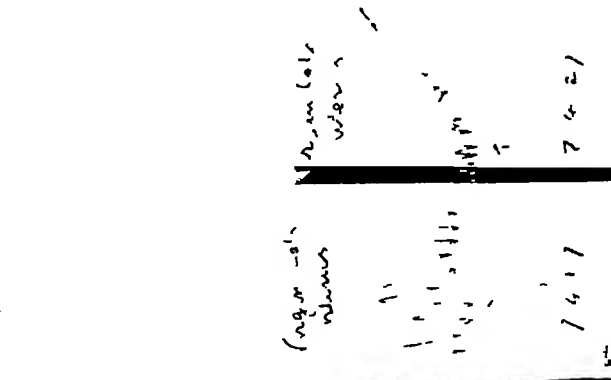
(c) Cat early pregnancy uterus in situ and BP 5 mgs I V quinine 1-300 000 Lower quinine 1-200 000



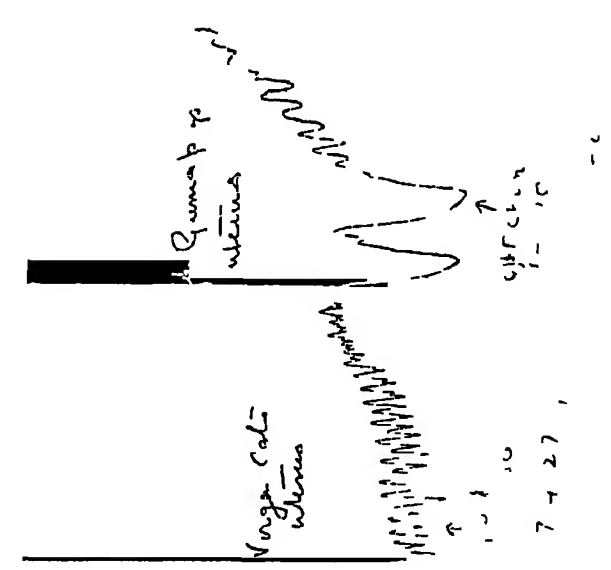
(d) Cat virgin uterus in situ and BP 5 mgs of Optochin I V, Urethane. Time-6 seconds



(e) Cat multiparous uterus in situ and BP 5 mgs of Optochin I V, Urethane. Time-6 seconds

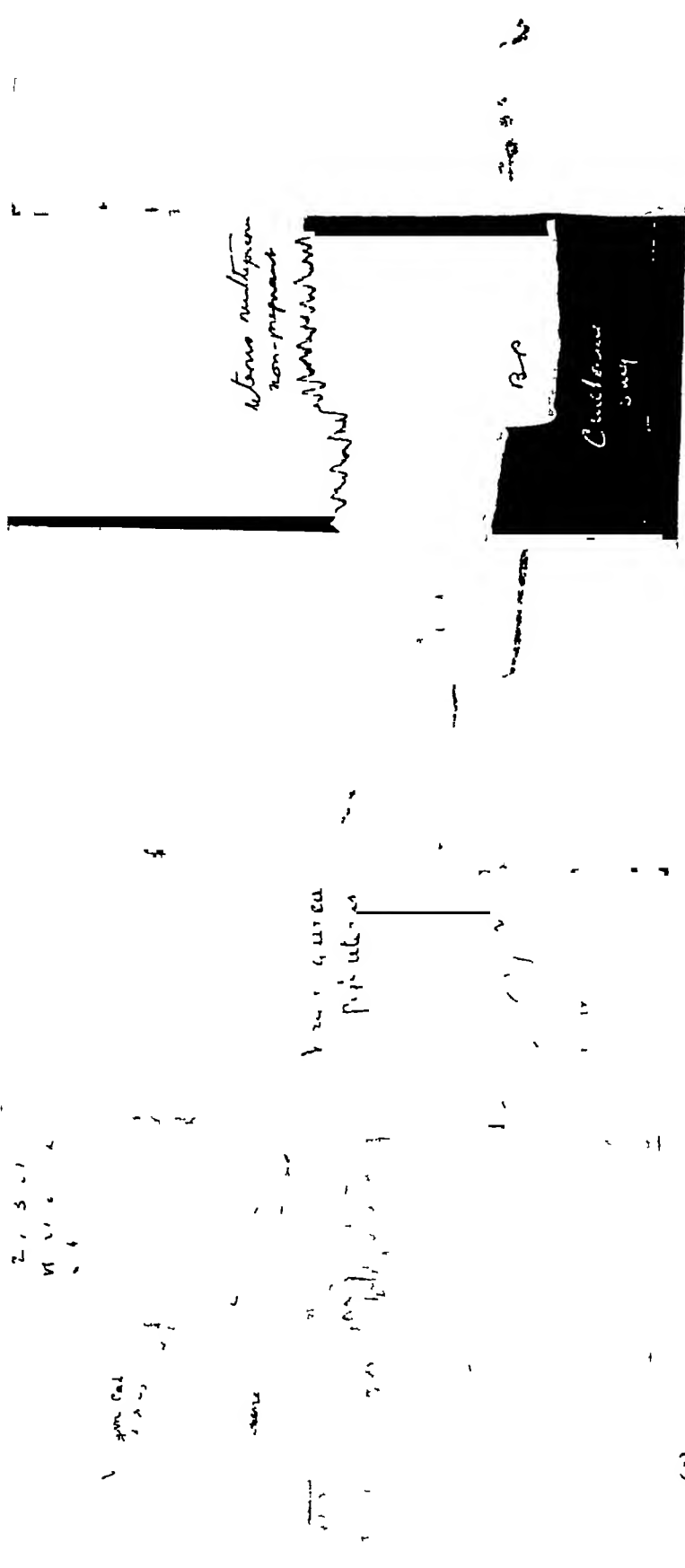


(f) Cat isolated uterus in situ and BP 5 mgs of Optochin I V, Urethane. Time-6 seconds



(g) Isolated pregnant uterus, upper quina mine 1-500,000 Lower quinine 1-100,000

# GRAPH II



(a) Isolated virgin cat's uterus  
Upper 1—200,000  
cinchonidine pH 7.6  
Lower, 1—100,000  
dilution pH 7.2

(b) Isolated uterus  
Upper, Multiparous cat's 1—20,000  
of cinchonidine  
Lower Virgin  
guinea-pig's  
1—150,000 of cinchonidine

(c) Isolated uterus virgin  
guinea-pig, cinchonidine  
1 in 200,000 at arrow

(d) Cat, virgin, uterus in situ,  
and B P cinchonine 5 mgs  
I V

(e) Cat, multiparous, uterus in  
situ and B P 5 mgs of cinchonine I V  
Urethane and  
Ether, Time—6 seconds

(f) Cat, advanced pregnancy Uterus in situ and B P cinchonine 5 mgs I V

We have found that when quinine is given intravenously in 5 mg doses to pregnant cats under anæsthesia weighing 2 to 2.5 kilos, a powerful tonic contraction is produced if the pregnancy is far advanced, but in early pregnancy no such tonic contraction is produced. In this also we confirm Acton's findings (1921). He found that quinine in dilutions of 1 in 150,000 increases the strength of the intermittent uterine contractions and in the presence of a predisposition or an exciting cause, the pressure exerted might be sufficient to cause rupture of the membranes or dilatation of the os and so bring on labour. Our experiments show that the oxytocic action of quinine is quite pronounced when the uterus is nearly full term or when labour is on, but in early cases of pregnancy, beyond slightly increasing the amplitude of the automatic movements no untoward effect is likely to be produced.

With regard to quinidine the literature cited is most confusing. While some workers like Zanda (1910) obtained only relaxation, others like Worth Hale (1916) have claimed that this alkaloid was about 10 times as strong as quinine in its stimulating effect on the uterus. We have also been faced with some apparently contradictory results during the course of our experiments, but we have been able to draw some general conclusions. On the virgin uterus in situ, a relaxation sometimes preceded by a contraction is produced, on the pregnant uterus under the same conditions relaxation is never produced, but on the other hand both the tone and the rhythm are improved, especially when the pregnancy is advanced. This relaxation never occurs in the isolated organ and it is difficult to understand why it is obtained only in the virgin uterus in situ.

Quinidine is slightly more powerful than quinine on the isolated uterus, both pregnant and non-pregnant, but quinine has a decidedly stronger action on the pregnant uterus in situ. In agreement with the findings of Nelson and Thomas (1927), no experimental data have been obtained by us to show that quinidine might be more effective as an oxytocic than quinine and we are therefore unable to confirm the finding of Worth Hale that quinidine is ten times stronger than quinine.

Iso-octyl hydrocupreine presents some features similar to quinidine. It has a very marked action on the intact and isolated multiparous and pregnant cat's uterus, causing tonic contractions. In the virgin uterus in situ, however, relaxation is produced preceded by a small initial contraction. Vuzin appears to have a toxic action on the animal which is as marked as that of quinidine, a great fall of blood pressure is produced in both cases followed sometimes by convulsions. This toxic action may possibly be a contributory factor in causing relaxation.

Ethyl-hydrocupreidine produces marked tonic contractions of the cat's uterus, both in the pregnant as well as the non-pregnant states. On the isolated uterus of the guinea-pig also similar marked effects are noticed even in dilution as high as 1 in 200,000. But curiously enough, hardly any noticeable effect is produced on the isolated uterus of the cat even in such high strengths as 1 in 20,000 within a range of pH from 7.2 to 7.6. The cause of this discrepancy is not understood.

from 5 to 10 mgs. On the virgin intact uterus, 5 mgs produce a well-marked relaxation, sometimes preceded by an initial contraction (Graph IV *a*). In some of the animals we noticed that the respirations stopped after the drug was administered, at the same time there was marked relaxation of the uterus, the breathing could not be revived even after continued artificial respiration. In the multiparous non-pregnant uterus this relaxation effect is not observed so often, nor is the initial contraction marked (Graph IV *b*). The uterus in early pregnancy shows a well-marked contraction after injection of quimidine which is not tonic in character (Graph IV *c*), in advanced pregnancy there is improvement in both the tone and the rhythm of the contractions (Graph IV *d*).

On the excised virgin uterus, quimidine produces an increase in the amplitude of the contractions in dilutions of 1 in 150,000 at pH of 7.2, when the pH is changed to 7.6, 1 in 200,000 dilutions cause a marked tonic contraction (Graph IV *e*). With higher concentrations these effects become more marked (Graph IV *f*). On the multiparous non-pregnant and the pregnant uterus 1 in 100,000 to 1 in 50,000 concentrations increase the amplitude and rhythm of the automatic contractions (Graph IV *g*).

*On the guinea-pig's uterus*—The virgin uterus shows tonic contractions with dilutions of 1 in 75,000 to 1 in 20,000, the effect becoming more and more marked as the strength of the solution is increased (Graph IV *h* and *i*). Higher dilutions have no marked effect. The pregnant uterus shows definite increase of tone and rhythm with dilutions of 1 in 100,000 (Graph IV *j*). Higher dilutions have little effect while stronger concentrations produce a marked tonic contraction of the organ (Graph IV *k*).

### Quinamine

(See Graph V)

*On the cat's uterus*—The action of quinamine on the uterus has been fully dealt with in a paper by Chopra and David (1927). It was shown that the virgin uterus of the cat in situ shows a well-marked tonic contraction when quinamine is given intravenously in doses of 3 to 5 mgs (Graph V *a*). If the uterus be relaxed with adrenalin and an injection of a few milligrams of the alkaloid given, not only is the normal tone regained but a tonic contraction is also produced. The reaction of the multiparous uterus to quinamine is very similar to that of the virgin uterus, both the tone and the automatic movements being markedly increased (Graph V *b*). The tone as well as the rhythmic movements of the pregnant uterus of a cat in situ are increased by 2 mgs given intravenously, while 4 to 5 mgs produce a well-marked tonic contraction of the uterus (Graph V *c*).

The isolated virgin uterus of the cat is stimulated with dilutions as high as 1 in 300,000 and a tonic contraction is produced, with stronger concentrations the effect is more marked (Graph V *d*). On the excised multiparous uterus a solution of 1 in 500,000 produces a marked increase in the tone of the organ. With higher strengths like 1 in 200,000 and more the effect is increased (Graph V *e*).

*On guinea-pig's uterus*—On the isolated virgin uterus of the guinea-pig, dilutions as high as 1 in 400,000 produce a well-marked tonic contraction, while

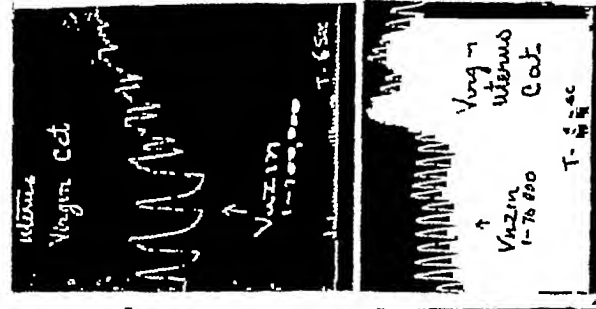
GRAPH IX



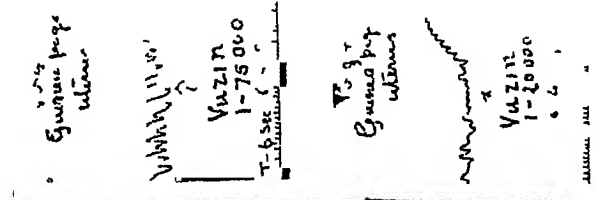
(c) Female cat, virgin, uterine movements and blood pressure. Urethane and Ether anesthesia Time—6 secs Vuzin 5 mgs intravenous



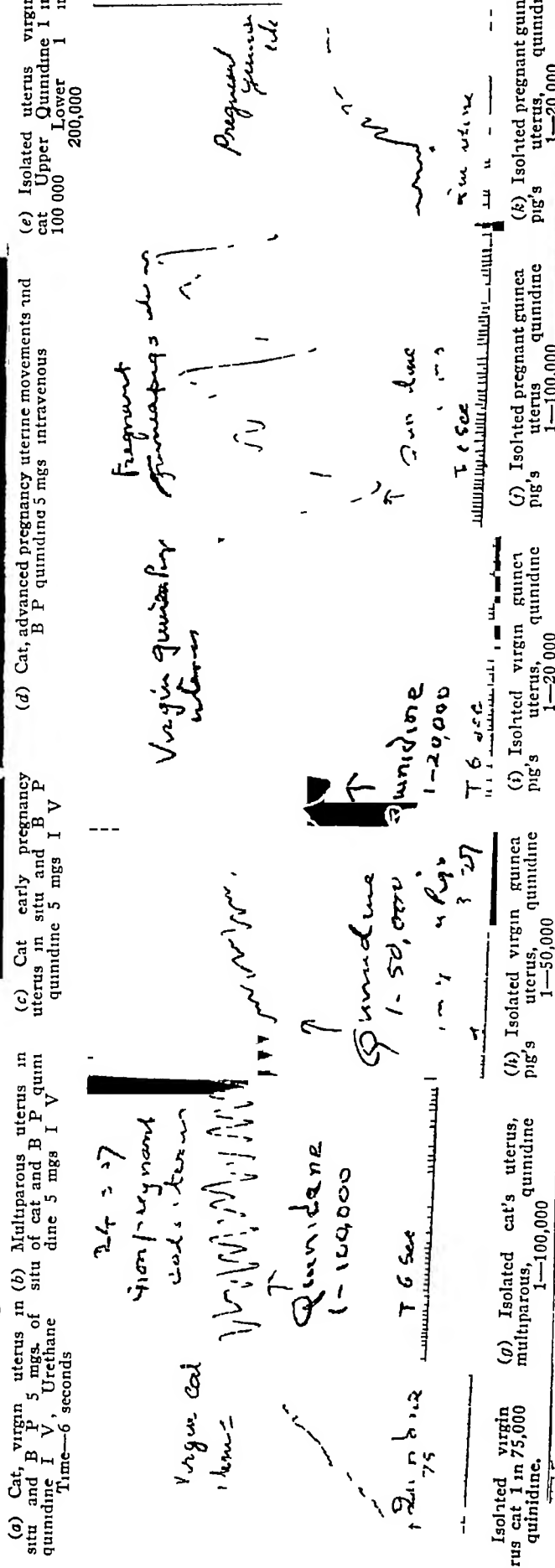
(d) Female cat pregnant uterine movements and B.P. Secondary rise of B.P. due to asphyxia Urethane anesthesia Time—6 secs Vuzin 5 mgs intravenous



(e) Isolated virgin uterus of cat Time—6 seconds Upper Vuzin 1—100,000 Lower Vuzin 1—75,000



(f) Isolated uterus virgin guinea pig Time—6 seconds. Upper Vuzin 1—75,000 Lower Vuzin 1—20,000





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(1926) p 248
- NELSON, E E and THOMAS, F W A comparison between the action of quinine and  
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uterus *Arch Intern de Pharmacod*, XXXII,  
455—60 (Abstract in *Physiological Abstracts*),  
Vol XII, No 3, June, p 181
- CHOPRA, R N and DAVID, J C 'The pharmacological action of quinamine' *Ind*  
(1927) *Jour Med Res*, Vol XV, No 2

slight increase in the amplitude of contractions in the case of the virgin uterus in situ. The non-pregnant multiparous uterus reacts with a tonic contraction (Graph VIII d). The tone of the pregnant uterus is slightly increased (Graph VIII e).

On the isolated uterus of both cat and guinea-pig the effect is the same as with iso-heptyl hydrocupreine (Graph VIII f and g).

#### *Iso-octyl hydrocupreidine*

(See Graph IX)

*On the cat's uterus*—Doses of 5 to 10 mgs intravenously produce a well-marked relaxation of the virgin uterus in situ, sometimes preceded by an initial contraction (Graph IX a). rarely relaxation is not observed but a tonic contraction is produced (Graph IX b). A pregnant uterus in situ always reacts with a marked increase of tone (Graph IX c). Iso-octyl hydrocupreine is a toxic drug and sometimes produces stoppage of respiration, and death from asphyxia after intravenous injections (Graph IX d).

On the isolated organ, dilutions of 1 in 100,000 and under produce a marked increase of tone or tonic contraction (Graph IX e).

*On the guinea-pig's uterus*—1 in 100,000 dilutions stimulate the automatic movements of the isolated uterus without increasing the tone. Higher strengths from 1 in 75,000 to 1 in 20,000 produce a tonic contraction with diminished amplitude and accelerated rhythm (Graph IX f).

#### *Discussion*

Three points strike one on perusal of the above results. Firstly, the uterus of different species of animals show varying degrees of reaction to these alkaloids, the uterus of the guinea-pig is much more sensitive than the uterus of the cat, secondly, the results obtained after giving the alkaloids intravenously, on the uterus in the intact animals do not always correspond with those obtained on the isolated organs of the same species of animals, and thirdly, the hydrogen-ion concentration of the perfusing fluid is an important factor. We have observed that while the hydrogen-ion concentration at which the isolated guinea-pig's uterus gives maximal results is 7.2, in the case of the cat it is 7.6. These factors, in our opinion, account for the discordant results obtained by the various investigators.

From these experimental data we have gathered, some general conclusions can be drawn. All the cinchona derivatives tested, show varying degrees of stimulating effect on the uterus of both cat and guinea-pig, the dextro-rotatory alkaloids being more powerful than the lævo-rotatory ones. These results are in accord with the conclusion drawn by Acton (1922). Quinamine, which is dextro-rotatory, has been shown to be the most powerful. Ethyl-hydrocupreidine and cinchonine, which are also dextro-rotatory, have a much more marked action on the uterus than their lævo-rotatory isomerides, iso-heptyl hydrocupreine has little or no effect on the uterus, while iso-heptyl hydrocupreidine possesses a comparatively stronger action. Iso-octyl hydrocupreine, which is lævo-rotatory, is also quite powerful in stimulating the uterus but we could not obtain its dextro-rotatory isomeride. The least powerful of all these alkaloids is iso-heptyl hydrocupreine and then follow iso-heptyl hydrocupreidine and cinchonidine.

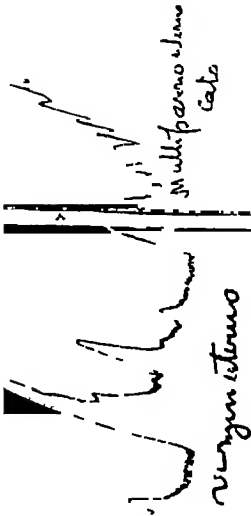
of sheep's red blood corpuscles by means of sodium glycocholate and taurocholate. The investigation was started not so much to obtain further data on the subject as to enable us, firstly, to get a better picture of the variation in the permeability of the stroma which occurs in presence of these various substances, and, secondly, to determine whether it is possible to inject suitable quantities of bile salts in order to effect the cytolysis of certain types of bacteria one of which, namely, *Pneumococcus* has been found to be rapidly cytolysed by these salts in test tube experiments. Obviously in animal experiments the blood serum may be expected to have a great effect on the cytolysis. This communication is concerned almost exclusively with the first point, reserving the cytolysis of *Pneumococcus* for the next one.

### EXPERIMENTAL

In the experiments given in the following pages, washed sheep's corpuscles, completely freed from serum by repeated centrifuging with normal saline was used. The red blood corpuscles were suspended in physiological saline. The solution of serum was prepared in the same saline in the dilution of 1:10. The egg albumin was obtained from a fresh egg and similarly diluted. The solution of bile salts was also made in normal saline and was M/20 strong. In actual experiment 2 c.c. of a 5 per cent suspension of the red blood corpuscles was taken in a test tube, and in another test tube, a known amount of the bile salt solution was mixed with a known amount of serum and a calculated amount of normal saline was added to it to bring the combined volume of the red blood corpuscle suspension and the bile salt solution when mixed together to a standard volume of 10 c.c. The contents of the two tubes were rapidly mixed three times and then allowed to rest in a stand. In the majority of the experiments an arbitrary time of 12 minutes was given for complete hæmolysis to take place which was determined visually by comparing with a standard hæmolysed tube. By varying the quantity of bile salt added, the exact amounts of either glycocholate or taurocholate which would hæmolyse completely the 2 c.c. of suspension in presence of known amounts of serum in 12 minutes were determined. When sufficient care was taken to use always sterilised flasks, test tubes and pipettes, the results were quite reproducible, and hence quite comparative also. The bile salt solutions were prepared fresh for each day's experiment. The experiments were done at the laboratory temperature of about 87° to 88°F. When not in use, the corpuscles were kept in an ice chest.

In Table I some data are given on the inhibitory effect of serum on glycocholate and taurocholate hæmolysis. The object was to make a comparative study of the hæmolytic powers of these two salts. As far as the authors are aware, this has not been done by any previous investigator. The study would be interesting however, because in the bile excretions, the amount of glycocholate is almost double that of taurocholate.

The following show quite conclusively that glycocholate has a much greater hæmolytic power than the taurocholate even in the presence of the blood serum. The inhibitory power of the serum even in very small quantities may also be noted. These facts are important in certain pathological cases such as in jaundice.

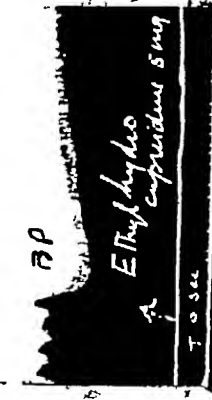


Female cat  
2370 grams  
urethane - 29 4 27

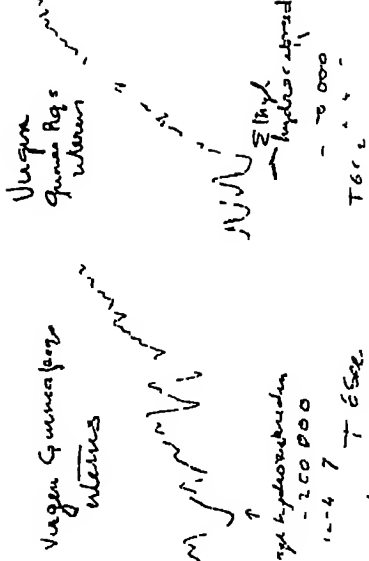
Bp

Bp

Ethyl hydrocupreidine 5mg  
11-6 27  
- 11-6 27  
- 11-6 27



(c) Cat, virgin uterus in situ (b) Cat, multiparous in situ and B P Ethyl hydrocupreidine 5 mg I V, Urethane B P Ethyl hydrocupreidine 5 mg I V Ether Time—6 seconds, cupreidine 5 mg I V



3 Cats shown

11-6 27  
- 11-6 27  
- 11-6 27

tone

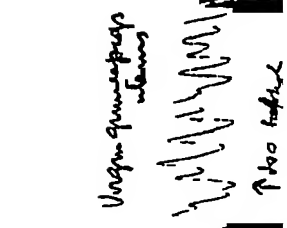
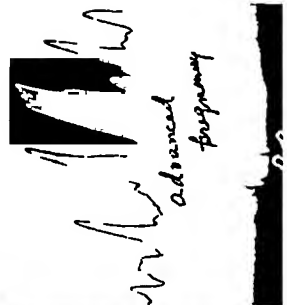
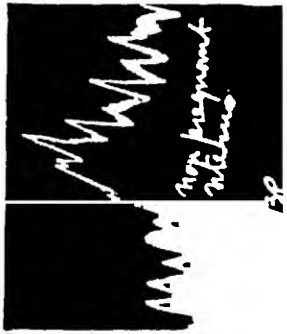
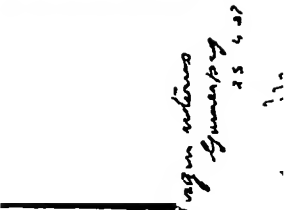
1000  
- 200000  
- 200000

tone

tone

(c) Isolated uterus virgin (f) Isolated uterus virgin guinea pig 1 in 200 000 guinea pig Ethyl hydrocupreidine 1 in 100,000

GRAPH VIII



virgin uterus  
- 25 4 27

advanced pregnancy

Virgin Guinea Pig uterus

1000

TABLE III

R B C = 2 c c

Total volume = 10 c c

Time = 12 minutes

Amount of egg albumin in c c	Amount of glycocholate required in grams	Amount of glycocholate neutralised per unit quantity of the egg albumin
0 0	01096	
0 1	02678	1682
0 2	06087	2495
0 3	08522	2475
0 4	10230	2283
0 5	12905	2362
0 6	14610	2252

The amount of glycocholate neutralised per unit quantity of the inhibiting substance which has been given in the third column of the above tables, has been calculated as follows. Taking for example the data with 0 5 c c serum, the excess of glycocholate necessary to hæmolyse the red blood corpuscles in presence of this amount of serum is 0 11201 to 0 01096 gram and hence the amount of glycocholate neutralised per unit of serum is  $\frac{0\ 11201\ \text{to}\ 0\ 01096}{0\ 5}$  gram. Other

data have also been obtained in a similar way. The results show the peculiar fact, that with both the inhibiting substances the amount of the hæmolyzing substance neutralised per unit of the inhibiting substance is almost constant, though the value of this constant differs in the two cases. Rejecting the first data, the mean value of the constant in the case of normal serum is 0 20008 and in the case of egg albumin is 0 2373. Egg albumin has thus a much greater inhibiting effect than normal serum.

It will be interesting to determine here if the amount of the hæmolytic substance neutralised per unit of serum remains a constant with increasing amount of the serum used. In that case the obvious conclusion would be that a chemical interaction occurs between the proteins of the serum and the hæmolytic substance. A careful scrutiny of the data with egg albumin shows that though approximately constant, the amount neutralised per unit of egg albumin tends to diminish with increasing amount of egg albumin. A similar fact was found by Ponder (1923) who observed that the quantity of the hæmolyzing substance neutralised per unit of serum decreases with increase in the concentration of the serum. A similar fact was also noted in our case with very high concentrations of the serum.

### Summary and Conclusions

1 The cinchona derivatives show varying degrees of stimulating effects on the uterus of the cat and the guinea-pig, the uterus of guinea-pig is much more sensitive than the uterus of cat. The pH at which maximal results are obtained varies with the uterus of different animals, the cat's uterus works best at pH 7.6 while the guinea-pig's uterus acts best at 7.2.

2 The dextro-rotatory alkaloids have a more powerful effect on the uterus than the laevo-rotatory ones.

3 The most powerful in this series is quinamine of which such high dilutions as 1 in 500,000 cause marked tonic contractions of the isolated virgin, multiparous non-pregnant and the pregnant uterus. 2 to 5 mg doses given intravenously produce a marked tonic contraction of the intact uterus both pregnant and non-pregnant, the amplitude of the rhythmic movements is also increased. Next in order may be placed ethyl-hydrocupreidine, iso-octyl hydrocupreine or vuzin and cinchonine, whose activity is about the same. Next comes ethyl-hydrocupreine and then quinine and quinidine, the last two being about equally effective. The least powerful of this series, in the order of their activity, are cinchonidine, iso-heptyl hydrocupreidine and iso-heptyl hydrocupreine, the action of the last two being slight and inconstant.

4 Our experiments show that the oxytocic action of quinine is quite pronounced when the uterus is nearly full term, but in early cases of pregnancy, beyond slightly increasing the amplitude of the automatic movements no untoward effect is likely to be produced. This is also borne out by clinical experience.

5 No experimental evidence has been obtained to show that quinidine is more effective as an oxytocic than quinine or that it is ten times stronger in its stimulating action on the uterus than quinine.

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It is well known that the stroma is composed mainly of albumin, lecithin and cholesterol, all of which are colloidal in nature. We assume that ordinarily the membrane is in the peptised condition and consists of granular solid particles, the interspaces of which are filled in by the adsorbed aqueous medium, an assumption exactly analogous to the structure of the copper ferrocyanide membrane. Hence water and many water-soluble substances can pass through the membrane. Aqueous solutions of metal salts are not, however, able to permeate easily because the stroma is polarised owing to the existence of an electrical charge. Only in those cases where salts can flocculate the membrane materials can a change in permeability be observed. Thus it has been found by us that uranyl salts can flocculate colloidal lecithin rapidly, and Mikwa (1924) has found that small amounts of uranyl acetate actually damage the structure of the cells as is shown by the increased sensitiveness to physiological saline. Bile salts, however, do not flocculate any of the sol, albumin, lecithin or cholesterol. On the other hand, both lecithin and cholesterol are strongly peptised by the bile salts and hence the observed hæmolysis with bile salts is undoubtedly due to the peptisation of the membrane. The impermeability of the membrane to sugars is, however, due probably to a different cause, namely, due to negative adsorption, a fact well known in the case of copper ferrocyanide membranes. So far as the electrical polarisation of the stroma is concerned, this fact introduces a factor of great importance in hæmolysis by coagulation. The slight negative charge on the membrane must first be lowered below a certain critical potential before hæmolysis can be observed. Since in acid solutions  $H^+$  ions and in alkaline solutions  $OH^-$  ions are the most active, it is apparent that acid solutions will increase the hæmolysis, whereas alkaline solutions up to certain concentrations of  $OH^-$  ions will increase the resistance of the stroma. This is exactly what has been found in practice. Thus Eggerth (1924) found that when washed sheep's corpuscle is suspended in solutions of pH 5.2 or more acid, they become progressively more electropositive, and this change is coincident with hæmolysis. In investigating the hæmolytic power of  $H^+$  and  $OH^-$  ions, Mond (1925) found that a slight decrease in pH from neutrality causes hæmolysis, but on the alkaline side of the neutral point, there is a wide zone of resistance. With high concentrations of  $OH^-$  ions, however, hæmolysis is again evident. Quite analogous to these results, small amounts of alkali peptise the copper ferrocyanide membrane and increase their resistance to coagulating agents, greater amounts of alkali completely dissolves the membrane, whereas acids coagulate it easily, and when present in small quantities, act as accelerators of coagulation by other salts.

These facts are therefore in favour of the view that the irreversible permeability of the stroma in presence of hæmolytic agents is due either to a coagulation or a peptisation of the membrane. So far as bile salts are concerned, their action on the membrane is one of peptisation. It may be pointed here that though this peptisation of the membrane has not been much discussed before, D'Herelle (1924) has strongly emphasised the coagulation point of view as an explanation of certain types of hæmolysis and some other colloidal reactions in the body, and Pickering and Taylor (1924) have shown the essential similarity in the phenomena of clotting of blood and hæmolysis.

# ON THE HÆMOLYSIS OF RED BLOOD CORPUSCLES BY BILE SALTS AND THE INHIBITING ACTION OF NORMAL SERUM AND EGG ALBUMIN, WITH REFERENCE TO THE PERMEABILITY CHANGE OF THE STROMA

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It is well known that certain substances like saponin, soaps and bile salts cause rapid hæmolysis of red blood corpuscles, but in presence of the blood serum this hæmolysis is retarded and in some cases altogether inhibited. Thus Ransom (1901) observed that cholesterol inhibits the action of saponin and he attributed the inhibitory action of serum to its cholesterol content. The inhibitory effect produced by serum on the action of bile salts was studied by Bayer (1907). It was found by him that cholesterol has no inhibitory effect, that lecithin produces inhibition, but not in quantities that occur in blood and that the proteins of blood are responsible for the inhibition. In this connection may be mentioned the observations of von Eisler (1906) who stated that serum globulin inhibits the action of staphylolysin and tetanolysin, and also those of Lieberman (1907) who found that hæmolysis by soaps is prevented by serum albumin. Bayer's researches have in the main been confirmed by Sellards (1908). In recent years quite a large amount of work has been carried out on this subject. Ponder (1923) has investigated the inhibitory effect of sera, sugars and other substances on saponin, soap and taurocholate hæmolysis. Certain substances are also known, such as acetic acid, aspartic acid, etc., which act just in the opposite way, namely, as accelerators of hæmolysis. These will be considered later on.

The present paper, which forms the first part of a series, deals with the study of the inhibitory action of blood serum and egg albumin in the hæmolysis





TABLE I

R B C = 2 c c

Total volume = 10 c c

Amount of normal serum added	TIME IN MINUTES REQUIRED FOR COMPLETE HÆMOLYSIS IN PRESENCE OF	
	Na glycocholate M/20 1 c c	Na taurocholate M/20 1 c c
0 0	1	42
0 05	2	
0 10	4	92
0 20	12	Partial hæmolysis only after 2½ hours
0 30	30	
0 40	69	

when there is a great secretion of bile salts which are absorbed in the blood. The inhibitory action of the serum counteracts the abnormal hæmolysis which would otherwise occur due to the excess of glycocholate secreted. In the following Tables II and III, we have summarised the quantitative data on the inhibition of glycocholate hæmolysis by means of serum and egg albumin.

TABLE II

R B C = 2 c c

Total volume = 10 c c

Time of complete Hæmolysis = 12 minutes

Amount of normal serum added in c c	Amount of glycocholate required in grams	Amount of glycocholate neutralised per unit quantity of the serum
0 0	01096	
0 1	02435	1339
0 2	04870	1887
0 3	07305	2069
0 4	09009	1978
0 5	11201	2021
0 6	13392	2049

*Description of Stained and Mounted Specimens*

In the appended table are given the measurements and relative lengths of the different parts of the four specimens examined

*The Body* is from 2.7 to 3.3 mm in total length. It is about 1.10 times the length of the wing and about 0.54 times that of the hind leg.

*The Antennæ* (Plate LV, figs 3 and 4) are shorter than in the male, the total length being about 2.50 mm. The IIIrd segment forms about 1/6th the total

*Phlebotomus newstead* ( ♀ ♀ ).

Specimens number		Measurements in millimetres				Ratios and formulæ
		1*	2	3	4	
BODY	Clypeus and head	0.430	0.430	0.414	0.457	$\begin{cases} 1.03-1.15 \times \text{wing length} \\ 0.53-0.56 \times \text{hind leg} \end{cases}$
	Thorax	0.700	0.700	0.614	0.743	
	Abdomen proper	1.743	1.600	1.428	1.928	
	Sup. clasper	0.186	0.171	0.200	0.157	
	Total length	3.06	2.90	2.67	3.30	
ANTENNA	Labium	0.300	0.285	0.285	0.328	$\begin{aligned} & \text{III}=\text{IV} \neq \text{V} \quad \text{IV}=\text{V}=\text{VI} \\ & \text{IV}+\text{V}+\text{VI} > \text{XII}-\text{XVI} \\ & \text{III} < \text{XII}-\text{XVI} \\ & \text{Antennal formula } \frac{2}{\text{III}-\text{XV}} \\ & \begin{cases} 5.7-5.8 \text{ times segment III} \\ 5 \text{ times segments XII}-\text{XVI} \end{cases} \end{aligned}$
	Epipharynx	0.286	0.271	0.257	0.300	
	Segment III	0.421	0.421	0.385	0.464	
	Segment IV	0.200	0.214	0.200	0.228	
	Segment V	0.193	0.200	0.200	0.228	
	Segment VI	0.186	0.200	0.185	0.221	
	Segments XII-XVI	0.482	0.471	0.471	0.514	
	Total length	2.40	2.40	2.28	2.67	
PALP	Segment 1	0.042	0.036	0.033	0.045	$\begin{aligned} & \text{Formula } 1(2, 1), 3, 5 \\ & = \frac{1}{3} \text{ of 3rd segment} \\ & \frac{P}{L} = 2.74-2.87 \quad \frac{P}{E} = 3.0 \end{aligned}$
	Segment 2	0.127	0.135	0.126	0.141	
	Segment 3	0.180	0.186	0.171	0.195	
	Segment 4	0.129	0.141	0.129	0.141	
	Segment 5	0.372	0.321	0.354	0.378	
	Total length	0.852	0.820	0.813	0.900	

\* Type female specimen

These facts naturally raise the important question of the nature of the action exerted by the inhibitory substances like proteins on the hæmolysis of the red blood corpuscles. Drawing an analogy from the properties of colloids, we know that many hydrophilic sols like albumin and similar proteins are good sensitising agents of other hydrophilic or hydrophobic colloids. Thus Brossa and Freundlich (1915) found that albumin sensitises a ferric hydroxide sol, and according to Brossa (1923) serum globulin considerably sensitises a congo-red sol. Reitstotter (1920) has found that albumins from various sera sensitise strongly many sols. Michaelis and Rona (1907, 1908) have shown that sols of ferric hydroxide, mastic emulsion or kaolin suspension can be sensitised by natural protein solutions. In this class belongs the sensitisation of suspensions of bacteria by the blood plasma of pregnant women (Lowenthal and Berthau, 1919). In all these cases the sensitising action of the proteins is due to their adsorption on the surface of the colloids, and the assumption is therefore justifiable that in the case of blood corpuscle also the action of normal serum is one of sensitisation of the colloidal membrane. The agglutinating power of different cations are according to Gough (1924) in the case of sheep's corpuscles in the order  $Ce > Th > Ca > K_4 Fe (CN)_6$  whilst the coagulating power of these cations on a copper ferrocyanide sol as determined by us is  $Ce > Th > Ca > K_4 Fe (CN)_6$  beginning with the highest. Copper ferrocyanide further offers a closer analogy in that it forms membranes with properties very similar to those of the stroma. Thus it is impermeable to sugars, and to many metal salts at low concentrations, which is quite analogous to the behaviour shown by the stroma. A recent investigation by Gurchot (1926) has shown that the variable permeability of the copper ferrocyanide membrane in presence of different substances is caused by the coagulation or peptisation of the membrane, and he has drawn attention to the fact that the change in permeability of many plant cells in presence of alcohols investigated by Czapek (1910) and of other membranes in presence of different organic acids investigated by Walden (1893) is nothing but due to a coagulation of the membrane materials. If now a similar view is advanced to explain the permeability of the stroma, the following becomes obvious. Any substance which will strongly coagulate the membrane so as to form cracks or holes will increase the permeability of the stroma to hæmoglobin and similar will be the effect of those substances which will strongly peptise the membrane particles thus loosening the whole structure. The equilibrium condition of the membrane is thus the stage where the membrane is neither too strongly coagulated nor too strongly peptised. Since in the case of bile salts it has been found by us that these substances have a great peptising influence on the lecithin and cholesterol parts of the membrane, it appears that in the action of bile salts, the hæmolysis is the outward sign of the irreversible peptisation of the stroma. Naturally the sensitising agent will antagonise the peptising agent, and hence the inhibiting effect of the proteins is only a special case of the antagonistic action of two substances well known in colloids, biochemistry and physiology (Sen, 1926, Clowes, 1916, Lillie, 1912, Osterhout, 1913, 1914). That the permeability change of the stroma in presence of the bile salts is due to peptisation will be evident from the following considerations.

leg and its breadth is approximately equal to  $\alpha$ . The other points of importance are that  $\alpha$  is about twice the length of  $\beta$  and is slightly longer than  $\gamma$ , the ratio  $\delta$  over  $\alpha$  is about 0.22 and the proximal fork of the 2nd vein is much nearer the base of the wing than is the fork of the 4th,  $\tau$  being about 0.12 mm.

The Halteres (Plate LV, fig. 5) has not the curious irregular appearance seen in the male (Sinton, 1926). It is more like the same structure in other species of *Phlebotomus*, but has not such a bulbous end.

The Hind Leg measures about 4.8 to 5.8 mm in length, being relatively very long. The femur forms about 1/5th of its total length and is about equal to the combined lengths of segments 2 to 5 of the tarsus. The tibia is almost twice the length of the femur. The 1st tarsal segment equals about 1/4th the total length of the leg.

The Female Genitalia (Plate LV, figs. 7, 8 and 9). (a) The spermatheca (Plate LV, fig. 7) is of the 'crenulated' type seen in the other members of the 'erect-haired' group (Sinton, 1927). It has a characteristic long, thin and worm-like appearance, which is distinctly different from the shape of this structure as described in any of the other Indian sandflies (cf. Sinton, 1927, Plate VII). (b) The postgenital ridge (Plate LV, fig. 9) has no large hairs or spines, but is covered with numerous fine hairs, as in *P. papatasi*. The chitinous plate of the ridge shows a tendency to be divided into two parts, as in the species just mentioned. (c) The furca (Plate LV, fig. 8) more nearly resembles that of *P. papatasi* than that of any other Indian species (cf. Sinton, 1927, Plate VII).

#### *Identity of Specimens*

One specimen was part of a catch of one male and three female *Phlebotomus* collected at the same time in a bed-room. The male was *P. newsteadi* and the two other females *P. major*. The co-types were caught in places where males of *P. newsteadi* had been taken.

The various ratios, formulæ, etc., are practically identical with those found in the male of *P. newsteadi* and have no very close resemblance to those of *P. major*, with which it might be confused on superficial examination. The only other erect-haired species recorded among thousands of *Phlebotomus* collected in this area, are *P. major* and *P. simillimus*. The specimens described differ from these species in the curious shape of their spermathecae as well as in many other points. The fact that one specimen, at least, was caught along with a male of *P. newsteadi* and the close resemblance of the formulæ, etc., makes it highly probable that this is the hitherto undescribed female of this species.

#### *Diagnostic Features*

In the fresh state the abundant and very markedly erect hairs on the dorsum of the abdomen, as well as its large size and very long legs, distinguish it from *P. simillimus* and *P. christophersi* in which these features are not marked.

The only other 'erect-haired' species which has been found in the same area as *P. newsteadi*, is *P. major*. It can be differentiated from this species in the fresh state by its much narrower wings and possibly by the position of the forking of the 2nd and 4th veins, which in *P. major* are at nearly the same level.

## SUMMARY

(1) An experimental study has been made on the inhibitory effect of blood serum and egg albumin on glycocholate and taurocholate hæmolysis of sheep's erythrocytes

(2) It has been found that glycocholate is a much stronger hæmolysing agent than taurocholate

(3) The view has been put forward that hæmolysis by bile salts is the visible sign of an irreversible peptisation of the stroma. In the normal condition, the stroma is in a slightly peptised state. Blood serum inhibits the hæmolytic effect by increasing the coagulability of the stroma, and it is a special case of the general antagonism between a coagulating agent and a peptising agent in diphasic systems

The present work has been carried out in the Bacteriological Department of the Bactro-Clinical Laboratory Ltd, Calcutta, and our best thanks are due to the management for the necessary supply of the materials

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DESCRIPTION OF PLATE LV  
*Phlebotomus newsteadæ* ( ♀ )

- Fig 1 Wing  
„ 2 Palp N the 'curious spines of Newstead'  
„ 3 Segments XIII—XVI of antenna  
„ 4 Segments II—IV of antenna  
„ 5 Haltere  
„ 6 Base of the pharynx  
„ 7 Spermatheca  
„ 8 Furca  
„ 9 Post-genital ridge

# NOTES ON SOME INDIAN SPECIES OF THE GENUS *PHLEBOTOMUS*.

## Part XXII.

### THE FEMALE OF *P. NEWSTEADI* SINTON 1926

BY

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WHEN the male of *P. newsteadi* was originally described (Sinton, 1926) the female of this species was unknown. However, a search amongst the other specimens of *Phlebotomus*, which had been caught in the same room about the same time as the type specimen, has revealed the presence of four females of this species which had not been differentiated from *P. major*. One of the specimens was caught at the same time as a male of *P. newsteadi* and two others in the same place about a month later.

It is probable that more specimens of this species have been collected in Kasauli but have been confused on superficial examination with *P. major* (♀). It had been thought probable that the unknown female of *P. newsteadi* might show the curious halteres so characteristic of the male of the species, but this is not the case.

The type female specimen was caught in a bed-room in Kasauli (6,000 feet) in the Himalayan foothills of the Punjab on 22nd April, 1921. Two other specimens were caught a month later and the fourth specimen in June of the same year.

#### *PHLEBOTOMUS NEWSTEADI*, (♀)

##### *Description of fresh specimens*

This is a very large insect in comparison with other 'sandflies'. Its general colour is light yellowish and the black eyes are in marked contrast to the rest of the insect. The integument is yellowish. The abdominal hairs are markedly erect and of a yellowish colour. The wings are narrower than in *P. major* and are covered with yellowish hairs.





*Phlebotomus newstead* ( ♀ ♀ ) —Contd

Specimens number		Measurements in millimetres				Ratios and formulæ
		1*	2	3	4	
Wing	Length	2 785	2 800	2 457	2 857	= 4 3X breadth, = 0 50X hind leg
	Breadth	0 643	0 657	0 570	0 657	$\frac{a}{\beta} = 1.94-2.14$ $\frac{\beta}{\gamma} = 0.55-0.57$
	$\alpha$	0 628	0 643	0 528	0 671	
	$\beta$	0 314	0 300	0 271	0 314	$\frac{\delta}{\alpha} = 0.20-0.23$ $\frac{\alpha}{\gamma} = 1.11-1.18$
	$\gamma$	0 543	0 528	0 471		$\frac{a}{\epsilon} = 0.72-0.78$ $\frac{\theta}{\epsilon} = 1.45-1.51$
	$\delta$	0 128	0 143	0 114	0 157	
	$\epsilon$	0 800	0 857	0 728	0 885	$\frac{\alpha + \beta}{\theta} = 0.72-0.77$
	$\theta$	1 214	1 243	1 100	1 328	$\frac{\theta}{\text{wing length}} = 0.43-0.46$
	$\pi$	0 143	0 157	0 100	0 143	
Hind Leg	Femur	1 085	1 057	0 914	1 114	= $\frac{1}{2}$ of leg
	Tibia	2 043	2 043	1 757	2 185	= 2 X femur
	Tarsus, seg 1	1 343	1 314	1 128	1 400	= $\frac{1}{4}$ of leg, = $\frac{5}{8}$ of tibia
	Tarsus, segs 2-5	1 043	1 043	0 943	1 100	$\overline{\overline{\vee}}$ femur
	Total length	5 51	5 45	4 74	5 80	(not including coxa and trochanter)

\* Type female specimen

length of the structure, and its tip extends as far as the distal end of the proboscis. The IVth, Vth and VIth segments are approximately equal in length and each is about half the length of the IIIrd segment. The IIIrd segment is shorter than the combined lengths of segments XII to XVI. The geniculate spines are paired and usually asymmetrically placed. The antennal formula is 2 over III to XV.

*The Palps* (Plate LV, fig 2) are about 0.85 mm in length. The formula is 1 (2, 4), 3, 5. The second segment is about  $3\frac{1}{4}$ th the length of the 3rd. The 'curious modified spines' described by Newstead are about 20 in number and situated on the basal third of the 3rd segment. The ratio palp to labium is about 2.8 and of palp to epipharynx about 3.0.

*The Pharynx* (Plate LV, fig 6). Adler and Theodor (1926) consider that the shape and arrangement of the 'teeth' at the base of this structure are of considerable diagnostic importance, especially in species of the *minutus* group. In *P. newstead* (♀), these 'teeth' are well-developed and numerous.

*The Wings* (Plate LV, fig 1) are long and comparatively narrow, the ratio length to breadth is about 4.3. The wing is about half the length of the hind

Boye (1926) tried this new drug on 6 cases of quartan malaria and found that it was more active against the clinical manifestations of this disease than was an equal amount of quinine hydrochloride

Marchoux and Quilici (1926) administered the drug to a case of malignant tertian malaria in doses of 0.25 gm twice daily and reported that it made the fever disappear in 3 days. Raynal (1927) also treated a case of malignant tertian malaria with stovarsolate of quinine. His treatment consisted of 0.5 gm of the drug night and morning for 10 days followed by a rest of 5 days and so on until four courses were completed, i.e., a period of nearly 2 months. This case was observed by blood examination during treatment but apparently not at a later date. He records a marked diminution in size of the spleen, a great improvement in the general condition and the disappearance of parasites from the peripheral blood.

Very few experiments with this drug have been recorded. No observations seem to have been made on benign tertian malaria nor on the effects of the drug in preventing relapse.

#### DRUGS USED IN OUR RESEARCH

Encouraged by the effects of stovarsol in destroying *P. vivax* in the peripheral blood, it was thought that possibly a combination of this drug with quinine might be more efficacious than the uncombined drug, in preventing relapses in chronic benign tertian malaria. At our suggestion the Haco Company of Berne through their agent Mr P. Bally, very kindly undertook the manufacture of such a compound, which was received by us in May 1926. The chemist of the Haco Company states that the drug is a well-defined chemical body of constant melting point, containing 52.6 per cent stovarsol and 47.44 per cent quinine. The drug was manufactured in pills with albuminates. Treatment with this drug was commenced early in June 1926.

Later in the year we were asked by Messrs May and Baker, London, to test a 'quinine-stovarsol' compound said to contain 50.2 per cent quinine and 40 per cent stovarsol. This compound has also been tried by us.

#### DETAILS OF METHODS, CONTROLS, ETC., USED IN THE TESTS

The details of the methods, etc., used in this work are similar to those already recorded in previous papers of this series.

#### TREATMENTS

As in our other work the usual preliminary purgation with calomel and magnesium sulphate was carried out. The patients were kept in bed for the first two days of treatment and the majority were fit for discharge from hospital at the end of the third day.

1. *Treatment S Q P*—The drug used in this treatment was the 'quinine-stovarsol' of the Haco Company. Eight pills were given at 10 a.m. and a similar dose at 3.30 p.m. daily for 28 days. This corresponds to a daily dose of about 0.52 gm (8 grains) of stovarsol combined with about 0.48 gm (7 grains) of quinine. The total amount of stovarsol given to each case was about 14.7 grms (277 grains) and of quinine about 13.3 grms (205 grains).

In stained and mounted specimens the characteristic shape of the spermathecae distinguish it from any of the other members of the 'erect-haired' group yet described (*cf* Sinton, 1927, Plate VI) The other points of diagnostic importance are (*a*) the palpal formula—1 (2, 4), 3, 5, (*b*) the long and comparatively narrow wing, in which the 2nd vein forks proximal to the 4th; (*c*) the shape of the furca, (*d*) the position of 'Newstead's spines' on the basal third of the 3rd palpal segment, not the middle third as in *P major*, (*e*) the tip of the IIIrd antennal segment reaching to the tip of the proboscis, and (*f*) the very long legs

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the remaining three patients, one completed an observation period of 7 weeks before he was lost sight of and the others did not relapse during observation. The relapse rate for this series was, therefore, a possible maximum of 84.6 per cent with an observed minimum of 76.9 per cent.

The relapses were detected at the following periods of observation after treatment—Four in the second week, three in the third, one in the fourth and two in the sixth.

2 *Treatment S Q*—Ten patients were treated in this series, and amongst these six relapses due to *P. vivax* were observed. The other four patients completed an observation period of 9 weeks without relapse. The observed relapse rate was, therefore, 60 per cent. The relapses were observed at the following periods of observation after treatment—Three in the third week, two in the fourth and one in the sixth.

#### *Results in the Control Groups*

1 *Treatment Q C M*—Of thirty-three patients 15 relapsed and one was lost sight of before the termination of observation. The relapse rate was an observed minimum of 45 per cent with a possible maximum of 48 per cent.

2 *Treatment M C M*—Of twenty-nine patients in this group the observed minimum relapse rate was 59 per cent with a possible maximum of 62 per cent.

3 *Treatment C C M*—Amongst twenty-three patients in this group the observed minimum relapse rate was 69.5 per cent with a possible maximum of 74 per cent.

4 *Treatment C D C M*—Amongst twenty-three patients the observed minimum relapse rate was 61 per cent with a possible maximum of 69.5 per cent.

#### DISCUSSION OF RESULTS

In the quinine-stovarsol series of 23 patients, the observed minimum relapse rate was 69.5 per cent with a possible maximum of 73.9 per cent as compared with 57.4 and 61.1 per cent in 108 controls of the cinchona alkaloid series. These results were better than those observed after a short course of intravenous injections of sodium stovarsol (Sinton, 1927) and are very similar to those obtained with quinine and stovarsol given separately (Sinton, 1926).

The quinine-stovarsol groups receiving 9.6 grains of stovarsol combined with 12 grains of quinine daily show a better result than those in which only 8 grains and 7 grains respectively of these two drugs were given. In neither case were the results as good as in the control series. It is possible that if the daily amount of quinine was greater, say 20 grains, or if larger initial doses of both drugs were used, the results might be better.

#### EFFECTS OF QUININE-STOVARSOL ON THE CLINICAL MANIFESTATIONS

##### *A Clinical Cure*

Quinine-stovarsol rapidly produces a clinical cure in benign tertian malaria. After the treatments described, parasites were absent from the peripheral blood.



Fig 2

0 50  $\mu$

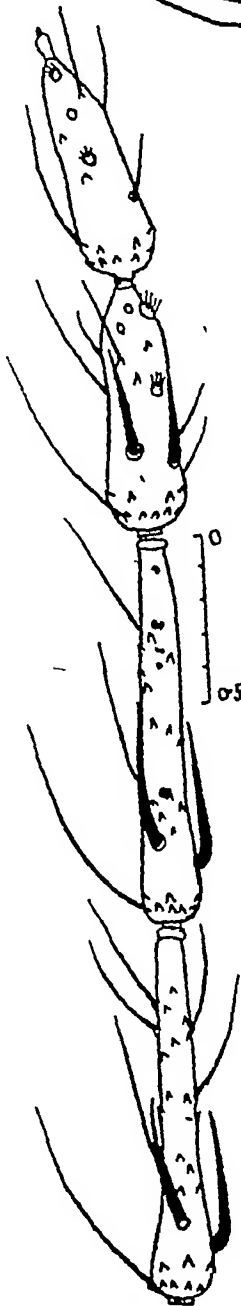


Fig 3



Fig 4



Fig 5

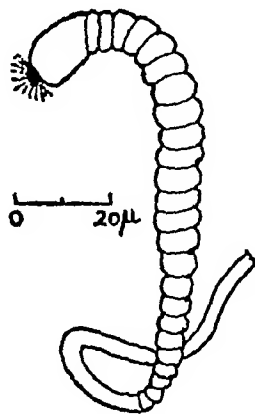


Fig 7

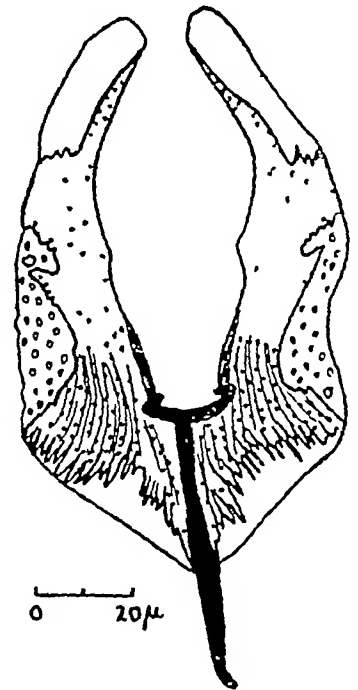
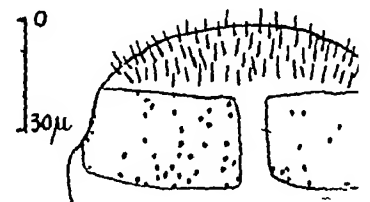
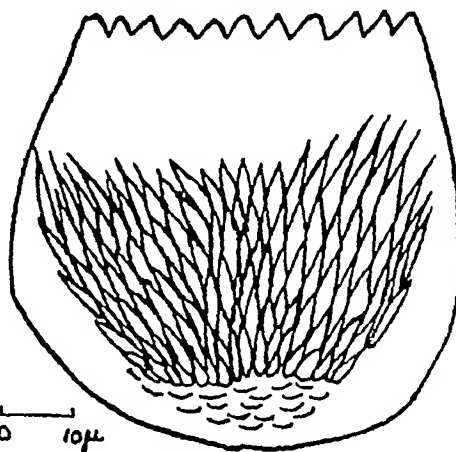


Fig 8



### Conclusions

Under the conditions of our work, stovarsol seems to be a useful adjuvant to quinine in the treatment of malaria. The compound 'quinine-stovarsol' does not seem to be more efficacious than the two drugs given separately, but is more easily administered.

It is possible that if larger initial doses of both quinine and stovarsol were given during the first week of treatment followed by a course similar to that described in this paper better results against relapse might be obtained, especially if the amount of quinine was increased.

### THANKS

Our thanks are due to the Haco Company of Berne and to Messrs May and Baker, Ltd, London, who so kindly provided us with the drugs tested in this work.

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# STUDIES IN MALARIA, WITH SPECIAL REFERENCE TO TREATMENT

## Part VIII.

### THE ORAL ADMINISTRATION OF QUININE-STOVARSOL IN THE TREATMENT OF CHRONIC BENIGN TERTIAN MALARIA

BY

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THE effects of the oral and the intravenous methods of administration of stovarsol in preventing relapse in chronic benign tertian malaria have been described in two previous papers of this series (Sinton, 1926, Sinton, 1927) These results show that stovarsol has a marked destructive action on *P vivax* in the peripheral circulation, at least, but, although it cures the clinical symptoms of the disease, it does not prevent subsequent relapses in the majority of cases It was thought that by combining the destructive action of quinine on the parasites with that of stovarsol better results might be obtained in the prevention of relapse That such results might be obtained seemed probable if, as believed, the smaller forms of *P vivax* are mainly destroyed by quinine while stovarsol has a special action on the larger ones

#### OBSERVATIONS BY OTHER WORKERS ON THE EFFECTS OF QUININE STOVARSOULATE

Stovarsol (acetyl-oxyamino-phenyl-arsenic acid) was originally prepared by Fourneau (Marchoux, 1925) Since that time, this worker has prepared a new salt of this drug—'stovarsolate of quinine'—which contains about equal amounts of quinine and stovarsol (Marchoux and Quilici, 1926)



- (ii) That cases sufficiently severe to merit much clinical attention were usually considered to be protozoal in origin, and along with many of the milder cases had in the past been treated with emetine
- (iii) That protozoal infections in reality were rare in comparison with the bacillary infections
- (iv) That many of the laboratory diagnoses in the past, in the light of modern knowledge, were probably incorrect and misleading. These findings were published in *The Journal of the Royal Army Medical corps* (6)

During the period between 1914 and 1925 considerable light has been thrown on the intestinal disorders of the East. Numerous observers have shown that, throughout the entire East, bacillary dysentery, particularly due to infections with the *B. flexneri* group, is extremely common. In India, Lieutenant-Colonel Cunningham, I.M.S. (3), has shown that in Madras and Bengal bacillary dysentery infections were frequent, and that a large percentage of the Indian population (22 per cent) suffered from 'latent bacillary dysentery,' i.e., a mild relapsing type, which from its very mildness is a danger from the carrier aspect.

Majors Acton and Knowles (4) showed that a similar high incidence of bacillary dysentery existed in Calcutta. These latter, and numerous other observers, had demonstrated the importance of the bacillary exudate as a microscopic method of diagnosis. The fact that the *B. flexneri* group was responsible for attacks of intestinal inflammations resulting in clinical manifestations varying from a mild diarrhoea to a severe attack of dysentery had been recognised. In 1919 the Medical Research Committee had published the work of Andrews and Inman (5) in which the various known strains of *B. flexneri* were collected, and placed in definite standard strains. Dreyer's method of agglutination and its advantages had been recognised. This method of agglutination had been brought into use wherever careful standardised methods of agglutination were carried out.

The general sanitary arrangements in Poona in 1925 appeared to be similar to those existing in 1913 when Colonel Morison carried out his investigations, with however, the important exception, that, as a result of Colonel Morison's work, what appears to be an efficient chlorination of the water supply, under careful bacteriological and chemical control has been instituted. The results of bacteriological examinations of the chlorinated water are recorded daily, and lactose fermenters are never present in 100 c.c. The writers have confirmed this on many occasions.

Cholera is practically unknown now in Poona city and the few enteric cases met with among the troops have been proved to be due to carriers. No suspicion of a water-borne epidemic has arisen.

In contrast to the above, the intestinal disorders still persist throughout the year, with approximately the same seasonal rise as in previous years. In the two years under review, however, there has been no suggestion of any epidemic, as apparently occurred in the past. For example, in August 1926, the worst

As a preventive of any of the possible toxic effects of the arsenical portion of the drug, each patient was given 4 grms (60 grains) of magnesium sulphate daily in the morning. In addition a dose of 60 grms (15 drachms) of sugar and 1 gm (15 grains) of sodium bicarbonate were given in solution with every dose of pills (Sinton, 1927).

2 *Treatment S Q*—In this treatment the 'quinine-stovarsol' of May and Baker was used. The first six patients receiving this treatment were given 3 pills at 10 a.m. and a similar dose at 3-30 p.m. for 28 days. As a very severe febrile reaction was noted in the patients receiving 6 pills on the first day, the dose was reduced to 3 pills on the first day, followed by the same dose twice daily for the next 27 days and one dose on the 29th day of treatment. Six pills correspond to about 0.64 gm (9.6 grains) of stovarsol and about 0.8 gm (12 grains) of quinine daily. The total amount of stovarsol received by each patient was, therefore, about 17.9 grms (269 grains) and of quinine about 22.5 grms (337.5 grains). The same precautions against arsenical poisoning were taken as in the last treatment.

3 *Control Treatments*—The same system of controls was used as in the previous work. (a) *Treatment Q C M*. The patients receiving this treatment were given the quinine sulphate, magnesium sulphate, and citric acid mixture already described (the 'Mist Q' of Sinton, 1926). The dosage was 30 grains (2 grms) of quinine daily for 2 weeks and 10 grains (0.66 gm) daily for a further 6 weeks. The total amount of quinine given to each case was 1,190 grains (77 grms). (b) *Treatment M C M*. These patients received 20 grains (1.30 grms) of a standardised 'cinchona febrifuge'—('Malarene,' *vide* Sinton, 1927) daily for 28 days. The total amount of cinchona alkaloids given to each patient was 560 grains (36.5 grms). (c) *Treatment C C M*. The mixture used was similar to the 'Mist Q' mentioned above, except that the quinine sulphate was replaced by cinchonine sulphate. Each patient received 20 grains (1.30 grms) daily for 28 days, a total of 560 grains (36.5 grms) per case. (d) *Treatment C D C M*. In these cases the cinchonine was replaced by an equal amount of cinchonidine sulphate and administered for the same time. The total amount of cinchonidine given was 560 grains (36.5 grms) in 28 days to each case.

#### EFFECTS OF TREATMENT IN PREVENTING RELAPSE

'Relapses' were diagnosed as heretofore by the finding of malarial parasites in the peripheral blood by the thick film method of examination, during an observation period of 8 weeks after the cessation of all treatment. Patients who did not 'relapse' during this period were considered as 'cured'.

Amongst the stovarsol cases the average number of previous relapses per patient was 5.2 as compared with 4.1 amongst the controls. The spleen rate before treatment in the stovarsol cases was 39.1 per cent, while amongst the controls it was 30.6 per cent.

#### Result in the Quinine-Stovarsol Groups

1 *Treatment S Q P*—There were 13 patients in this series, amongst whom relapses due to *P. vivax* were detected in ten during the observation period. Of

## INCIDENCE AND NATURE OF POONAITIS

*Results of Work in 1925*

This year was largely spent in finding out the nature of the intestinal disorders with which we had to deal. As stated above, it was soon evident that we were dealing chiefly with a mild type of bacillary dysentery, and not with a simple diarrhoea also that many specimens did not reach the laboratory from these cases. Considerable propaganda was necessary among hospital staffs, particularly subordinates, in order to break down the fixed traditions of former years. The laboratory staff had also to be trained in proper technique. More equipment had to be collected and proper media prepared, etc.

Table I gives the number of cases from whom specimens of blood and mucus were sent to the laboratory from the British and Indian military hospitals at Poona and Kirkee.

It will be noted that these specimens are divided into those showing a typical microscopic exudate of bacillary dysentery and those reported on as showing an indefinite exudate. The latter term is used throughout this report to define specimens in which only a few polymorphonuclear leucocytes were found under the microscope, careful search often being necessary. In many of these cases faeces were present in the specimen along with the mucus. These exudates were found in (a) very mild cases, (b) cases in the early stages of the dysenteric attack (c) cases from which specimens were sent to the laboratory for the first time about the 4th to 5th day after onset of symptoms, i.e., recovering cases. In addition, it should be pointed out that a laboratory is entirely dependent on the hospital personnel for the selection of a suitable sample for bacteriological examination, and usually this important factor is left to unskilled subordinates. If the entire specimens could have been sent to the laboratory, the number of indefinite exudates recorded and the negative results would have been fewer in number. From hospitals situated some miles from the laboratory the sending of entire specimens was naturally impossible. From many of these, however, *B. flexner* was isolated, and, whether or not, all cases were treated as bacillary in origin unless amœbæ were found, i.e., treatment was entirely by salines. All responded quickly to this treatment, and as will be seen later serological examinations showed that 50 per cent of this type of cases in 1926 gave a rising agglutination titre to *B. flexner*.

From the table it will be noticed that the numbers of British and Indian troops reported on as suffering from a true dysentery were identical at the end of the year. In view of Colonel Cunningham's work this seemed improbable, but the Indian troops pay less attention to these mild attacks of diarrhoea with blood and mucus, and do not report sick to the same extent as the more highly educated British soldier.

In 1926 the cases among the Indian troops exceeded the British.

Twenty-three cases only of amœbic dysentery occurred among British and Indian troops during the twelve months of 1925, in striking contrast to the numbers occurring in previous years.

for an average period of 4.8 weeks after the end of treatment with stovarsol as compared with 5.1 weeks in the controls. The average period of absence was about a week longer in the S Q series than in the S Q P, which is probably due to the larger amounts of the drug given.

### B Temperature

The terms 'fever,' 'duration of fever' and 'rigor' are used as defined previously (Sinton, 1927). *Duration of fever* The average 'duration of fever' amongst the quinine-stovarsol series was 0.37 day (maximum 1 day) as compared with 0.31 day (maximum 1 day) amongst the controls. The 'duration of fever' in treatment S Q P was slightly longer than in treatment S Q.

Amongst the quinine-stovarsol series 56.5 per cent showed no rise of temperature after the commencement of treatment, while the percentage was 52.8 amongst the controls. *Rigors* Among the stovarsol series 43.5 per cent developed rigors inside 24 hours after the commencement of treatment.

The smaller number of patients who developed rigors in this series as compared with those in the previous experiments with stovarsol (Sinton, 1926, Sinton, 1927) is probably due to the smaller doses of the drug used in this series.

### C Splenic Enlargement

Amongst the Stovarsol group the spleen rate was 39.1 per cent before treatment, 17.3 per cent at the end of 1 week's treatment and 17.3 per cent at the end of treatment (four weeks). Amongst the control group these percentages were 30.6, 10.2 and 7.4 respectively.

### THE PROVOCATIVE ACTION OF STOVARSOL

In previous papers of this series attention was drawn to the fact that stovarsol had an effect in provoking a febrile reaction and the probable cause of this was discussed (Sinton, 1927). Guérin, Borel and Advier (1927) have also produced evidence in support of such a provocative reaction. In the majority of their cases they noted that during the course of 18 hours after the primary administration of the drug a sharp rise of temperature occurred. If the temperature was taken hourly, the percentage of cases in which such a rise was observed was greater, for the rise was often of short duration. They also found that no aggravation of the febrile symptoms occurred after the injection of stovarsol into healthy persons, into persons suffering from diseases other than malaria, nor were such febrile symptoms present after injection in other forms of malaria than benign tertian.

In the present series of cases severe rigors were observed in the S Q treatment until the initial dose was reduced. The provocative action is apparently not so marked when smaller doses of the drug are given. Our previous work was with daily doses of 1 grm which caused a more severe febrile reaction.

No toxic effects were noticed in any of the cases recorded in this paper.

Table II gives the cases among troops and families showing definite bacillary exudates, and the nature of the dysentery organisms isolated. From 52.2 per cent of cases dysentery bacilli were isolated (65 *flexner* and 17 *shiga*).

TABLE II

*Cases from all sources showing definite bacillary exudates  
1st examination, 1925*

Month	No. of exudates	<i>B. flexner</i>	<i>B. shiga</i>
January	8	4	
February	11	2	2
March	1	1	
April	15	1	
May	12	6	1
June	26	6	4
July	27	10	1
August	23	15	5
September	6	6	
October	7	4	
November	8	6	2
December	13	4	2
TOTAL	157	65	17

The rise in the incidence of cases in the months of June, July and August is apparent from the above tables.

From our experience in this year it was evident that, provided specimens from all cases of diarrhoea were sent to the laboratory, we should have little difficulty in separating true dysentery from the diarrhoea and colitis of the past. It was particularly evident that cases among the Indian troops, and to a less extent among British, were in the habit either of not reporting sick at all or too late for a positive laboratory diagnosis, and that therefore we had not arrived at the true incidence of diarrhoea in 1925.

We found that once laboratory media and technique were good, the question of a positive or negative result depended not on the laboratory, but on the hospital concerned. If a keen medical officer or a keen assistant, or sub-assistant surgeon were in charge of the dysentery ward the number of positive results increased accordingly. In fact a change in the personnel of these wards could be invariably diagnosed in the laboratory by a sudden drop in the number of positive results. Assistant and sub-assistant surgeons should be instructed and constantly

# REPORT ON AN INVESTIGATION OF DYSENTERY AND DIARRHŒA IN POONA

## Part I.

### GENERAL FINDINGS AND EPIDEMIOLOGY

BY

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ASSISTED BY

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[Received for publication, July 28, 1927]

POONA during the monsoon period, has for many years been noted for its intestinal disorders. Various investigations in the past have been carried out in this connection notably by Lieutenant-Colonel J Morison, I M S (1) (2), the results and conclusions of whose work were published in 1915 and 1916. Colonel Morison's conclusions were in brief that —

- (a) It was impossible to separate the various intestinal disorders, varying from colic and dyspepsia to the most severe types of dysentery and choleraic diarrhœa, either clinically or bacteriologically, and, for the purpose of his report, all were included together under the term 'Poona diarrhœa'
  - (b) That though the prevalence of flies bore a close relation to the intestinal disorders in the early period of the monsoon, this was not so after the month of August, when flies rapidly diminished in numbers. Nor apparently were flies ever found to be carrying any organisms definitely known to be causative of intestinal disease.
  - (c) The diarrhœa was apparently more closely connected with the rainfall, and probably was due to pollution being washed into Lake Fife, and distributed by the Poona water supply some six days later.
- Early in 1925 it was realised —

- (1) That intestinal troubles were common among the troops apart from the monsoon period, and that much of the so-called diarrhœa was a mild form of bacillary dysentery, mainly caused by organisms of the flexner group.

TABLE III  
1926, January—September.

Month	BRITISH TROOPS						INDIAN TROOPS						TOTAL BRITISH AND INDIAN TROOPS					
	<i>E. histolytica</i>	Total bacillary dysentery cases	Definite bacillary exudate	Indefinite bacillary exudate	Number of dysentery organisms isolated	<i>E. histolytica</i>	Total bacillary dysentery cases	Definite bacillary exudate	Indefinite bacillary exudate	Number of dysentery organisms isolated	<i>E. histolytica</i>	Total bacillary dysentery cases	Definite bacillary exudate	Indefinite bacillary exudate	Number of dysentery organisms isolated			
January	2	7	4	3	3	1	8	6	2	6	3	15	10	5	9			
February		12	11	1	8		13	12	1	9		25	23	2	17			
March	1	12	8	4	7		14	10	4	2	1	26	18	8	9			
April		11	7	4	5		7	5	2	3		18	12	6	8			
May	2	13	8	5	4		15	10	5	6	2	28	18	10	10			
June		8	8		6	1	26	22	4	17	1	34	30	4	23			
July		29	21	8	15		32	24	8	16		61	45	16	31			
August	2	32	19	13	10	1	34	25	9	22	3	66	44	22	32			
September	1	18	11	7	7	2	19	10	9	7	3	37	21	16	14			
Total	8	142	97	45	65	5	168	124	44	88	13	310	221	89	153			

month of the year, there occurred among the military population 66 cases of bacillary dysentery, and 40 cases of diarrhoea. These numbers among a population of close on 8,000 individuals (British and Indian) can not be considered in the nature of an epidemic. It may also be pointed out that the above figures represent closely the 'true' number of cases occurring among the troops in the station, and that many of the dysentery and diarrhoea cases, in the absence of specific orders, would not have reported sick owing to the mildness of their attacks. In 1925 the numbers were only rather more than half the above, largely due to the absence of specific orders that all such cases were to report sick.

In view of the new facts which have come to light in the past ten years and the presumption that, if chlorination of the water supply was efficient, the existing dysentery and diarrhoea must be due to other factors than the water supply, it appeared that the time had come to reconsider the subject of the intestinal disorders in Poona and Kirkee.

The points we hoped to investigate were —

- (i) To separate true bacillary dysentery as far as possible, from simple diarrhoea due to other cases and to ascertain its true incidence among British and Indian troops
- (ii) To follow the seasonal incidence of the dysentery and its relation to the rainfall, humidity, and fly prevalence
- (iii) If the period of increased fly prevalence was proved to correspond with the period of increased dysentery among the troops, to examine as many flies as possible bacteriologically for the presence of dysentery bacilli
- (iv) To ascertain whether the latent bacillary dysentery which Colonel Cunningham had shown to be present among the Indian population was a source of danger to the troops
- (v) To make a serological study of the agglutination reactions of cases known to be suffering from bacillary dysentery (*shiga* and *flexner*), both in cases from which bacilli had been isolated in the laboratory and in cases where laboratory technique had failed to isolate the infective organisms (dysentery group)
- (vi) To study the serological reactions of the organisms of the flexner group isolated, with a view to finding whether they agreed with those classified by Andrews and Inman
- (vii) To study the agglutinin content of serum from normal individuals (British and Indian) to *B. flexner* and *B. shiga*

The results of our investigations are not as complete as we had hoped, but routine laboratory work had naturally to take first place, and much of the work has perforce been carried out in the little spare time available after the routine day's work had been completed.

The work ceased suddenly in October 1926, owing to the unexpected transfer of one of us to another station, and, as a result, much of the serological work remains uncompleted.



## DYSENTERY, DIARRHŒA CASE SHEET

Number	Rank	Name	Unit
Age	Date of onset	Date on which first reported sick.	

		DAYS OF DISEASE.										
		1	2	3	4	5	6	7	8	9	10	11
Number of previous attacks												
Number of courses of emetin												
Macroscopic appearance of stools	1 Blood present											
Colour.	2 Colour of blood											
	3 Presence of mucus											
Reaction	4 Presence of fæces											
History of onset (Vomiting,) etc	5 Reaction of stool											
Pain—Nature and site.												
Tenderness and site	Temperature Morning											
	Evening											
Tenesmus												
Any other prominent signs or symptoms												
Duration of illness												
Treatment.												
Suggested causative factor												
	1 Food.											
	2 Chill											
	3 Exhaustion											
	4 Any other cause											
Any similar cases among associates												

*Table V* shows the total numbers of bacillary dysentery and diarrhœa cases during the 8 months for British and Indian Troops combined. It will be noted that 61 per cent of cases were true dysentery and 39 per cent diarrhœa. As in former years, cases occurred in all months, quite apart from the rainy

TABLE V

	Bacillary dysentery	Diarrhœa
January	15	17
February	25	14
March	26	16
April	18	8
May	28	24
June	34	14
July	61	46
August	66	40
September	37	19
	61 per cent	39 per cent

TABLE I  
1925, Poona and Kirkee

Month.	BRITISH TROOPS					INDIAN TROOPS					TOTAL BRITISH AND INDIAN TROOPS				
	<i>H. histolytica</i>	Total bacillary cases	Definite bacillary exudate	Indefinite bacillary exudate	Number of dysentery organisms isolated	<i>H. histolytica</i>	Total Bacillary cases	Definite bacillary exudate	Indefinite bacillary exudate	Number of dysentery organisms isolated	<i>H. histolytica</i>	Total bacillary cases	Definite bacillary exudate	Indefinite bacillary exudate	Number of dysentery organisms isolated
January		3	2	1	2		5	5		1		8	7	1	3
February	1	8	8		2		3	3		2	1	11	11		4
March	1						1	1		1	1	1	1		1
April	1	12	10	2	1	2	8	5	3		3	20	15	5	1
May	1	11	6	5	3		6	4	2	4	1	17	10	7	7
June	1	22	13	9	7		15	7	8	2	1	37	20	17	9
July	2	20	11	9	8	1	18	11	7	4	3	38	22	16	12
August	6	9	7	2	7	1	16	13	3	11	7	25	20	5	18
September	1	3	2	1	3		4	4		4	1	7	6	1	7
October	2	3	1	2		1	6	5	1	5	3	9	6	3	5
November	1	3	2	1	1		6	6		6	1	9	8	1	7
December	1	1	1				7	5	2	2	1	8	6	2	2
TOTAL	18	95	63	32	34	5	95	69	26	42	23	190	132	53	76

Major J Dowse, M C, R A M C, specially noted the clinical aspect of the dysentery and diarrhoea among British children admitted to the family hospital — These cases fell into two fairly well-defined classes —

- (a) 'Green diarrhoea' cases, with little mucus, and rarely blood in the stools. Occasionally mucus was present in considerable quantities

This type of case was found mainly in very young children, i.e., under two years of age, and was considered by Major Dowse to be due *not* to *fleener* or *shiga* infections, but to faulty feeding causing excessive fermentation, etc., 'as a result of which various organisms, *B morgan* No 1, etc., are no longer inhibited and give rise to intestinal inflammation'. The bacteriological control of these cases was not accurate, however, owing to the difficulties encountered in collecting and despatching stools to the laboratory. If the stools could have been examined shortly after passage and in the early stages of the disease, possibly many would have been found to be due to one or other of the dysentery bacilli. In such cases the children's napkins were usually sent to the laboratory, and the specimens were frequently dry on their arrival.

- (b) Children over two years old showed symptoms in no way differing from the ordinary mild or severe bacillary dysentery found among adults. Most of these cases were *B fleener* infections.

Amœbic diarrhoea, or amœbic dysentery was never discovered among children after Major Dowse took over charge of the family hospital.

We actually did find '*E histolytica* cysts' once in the stools of a boy twelve years of age.

Another point which appears worth noting, was the regularity with which children suffering from dysentery, and treated in quarters, infected the mother, or other children in the same household in 3 to 4 days. No carriers were ever discovered among the servants. In two instances in which typing of *B fleener* was carried out, the strain of bacillus isolated was the same in the mother and child.

It would appear, therefore, to be indicated that all dysentery cases among children should be treated in hospital, and not in quarters.

From the above observations it is obvious that 'Poonaitis' is largely bacillary dysentery, and that the main infective agent is *B fleener*, *B shiga* taking only a minor part.

Of the 39 per cent in which no blood or mucus was seen in the laboratory, small numbers were found to be due to *B ætryck* (3), *B gartner* (1).

*B fleener* was isolated from 5 and was probably responsible for more, if the well known difficulty in isolating this organism apart from its presence in mucus be taken into consideration. *Giardia intestinalis* accounted for a certain number particularly among the British troops, and the remainder were probably due to the above three agents, plus indiscretions of diet, chills, etc.

The same types of cases occur throughout the year and increase definitely in the monsoon period.

Serological examinations on 101 individuals suffering from 'dysentery group' infections (i.e., cases with bacillary exudates, but no dysentery bacilli

kept up to the mark as to the importance of sending specimens quickly from dysentery cases to the laboratory, and also as to the correct portion of the stool which should be selected for despatch

Generous financial help was obtained from the Indian Research Fund Association and steps were taken to make a closer study during the year 1926. The initial arrangements carried out were as follows —

- (i) Orders were issued through the Officers Commanding units that all cases suffering from diarrhoea were to report sick at the earliest possible moment. Local arrangements were made with the various medical inspection rooms for the collection and despatch of specimens to the laboratory.
- (ii) Printed instructions were given to medical officers and assistant surgeons, pointing out the difficulties connected with laboratory examination of dysentery stools, and the various important details connected with the collection of specimens.
- (iii) Weekly returns of all cases reporting sick with diarrhoea were sent to the laboratory from each M. I. room in order that the number of specimens examined could be checked by the numbers reporting sick.
- (iv) Printed case sheets were issued to medical officers interested on which a complete record of the important signs and symptoms of dysentery cases could be briefly noted.
- (v) Arrangements were made for as many cases as possible to be investigated serologically by having blood withdrawn at the commencement of the disease, and on the 8th, 12th, 16th and 23rd day after onset of symptoms.
- (vi) Fly traps were distributed throughout the barracks by Major G. Wallace, A.D.H. & P., Southern Command, and a weekly record was requested from the M. O. in whose area they were placed.

#### RESULTS IN 1926 TO END OF SEPTEMBER

*Table III* illustrates the number of cases reported on as suffering from dysentery. By the end of September the numbers were 142 British Troops and 168 Indian Troops, a great increase on the numbers occurring in 1925. This increase was in the main due to the orders regarding all troops reporting sick at the onset of an attack of diarrhoea. In the 9 months there was a total of 310 cases of bacillary dysentery as compared with 190 in the 12 months of 1925. Only 13 cases of amoebic dysentery were encountered.

*Table IV* gives the distribution of bacillary dysentery and diarrhoea among the various units. On the whole in proportion to the numbers of individuals in the units the incidence was much the same throughout the cantonment. It was noted that the Indian Troops suffered less from diarrhoea than the British Troops, but, as was expected, gave a higher incidence of dysentery, probably due to relapses in cases suffering from the condition termed by Colonel Cunningham 'Latent Dysentery'.

# CHART II.

ECUND RABAD

LY SEPT  
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JULY SEPT NOV  
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EB PR JUN AUG

JUL SEP O  
UNE U DE

AN MAR  
FEB PR JUNE UL SE  
AUG

TABLE IV  
Numbers of cases of dysentery and diarrhoea among units in Poona and Karkee to end of August 1926

Unit	JANUARY		FEBRUARY		MARCH		APRIL		MAY		JUNE		JULY		AUGUST	
	Dysentery	Diarrhoea	Dysentery	Diarrhoea	Dysentery	Diarrhoea	Dysentery	Diarrhoea	Dysentery	Diarrhoea	Dysentery	Diarrhoea	Dysentery	Diarrhoea	Dysentery	Diarrhoea
(British)																
1st K S L I	3	1	4	4	1	7	5		3	2	1		8	9	8	8
1st R W K	2	2	3	1	1	3	3	1	1	2	3		4	10	8	10
R A Karkee	2		5	3	1	2	1	1	1	2			0	8	6	7
R A O C						2							2	2	1	
I A O C							1						4	1	1	
Staff and Departments	1		1	2		1	2		3		2	3	4	1	2	3
Other Ranks (British)			1			2			5	1	3		3	3	6	
(Indian.)																
2nd Lancers	4	1	2		4	1	1	1	4		5	1	5		4	2
3-16th Punjabis	1	1	1	1	4	2	2		1		5	1	5	1	0	1
1-15th Punjabis							1		2		1		3		2	1
11th I B T Company			3	2	1		1		4		6		3	2	4	3
Indian Hosp Corps			2	1	1				2	1	4	1	5	1	4	
R B S and M	2		2		3		1	1	2	1	1		2	2	6	
1-2nd B Pioneers								2							2	1
Other Ranks (Indian)			1		1		2			1	3		5		3	

7 Amoebic dysentery is a comparatively rare disease among the troops as contrasted with the prevalence of bacillary dysentery

### EPIDEMIOLOGY

The next step in the investigation was as far as possible to ascertain the source of infection, and the method by which infection was conveyed to the troops

For the description of the sanitary arrangements existing in Poona given below, I am indebted to Major G Wallace, O B E, R A M C, A D H & P, Southern Command

### POONA

Poona—consisting of Poona city, the cantonment, part of the suburban municipality and the railway area—is situated on the right bank of the Mutra-Mula River at a height of about 1,850 feet above sea-level

The ground is undulating from east to west with drainage to the north towards the river by means of several nullahs

The city is generally lower than the cantonment

The soil consists of stratified trap rock which frequently outcrops on the surface but in other places is covered by hard modrum

### CLIMATE

The hot weather lasts from March till the end of May, the maximum temperature reached being about 110°F in May

The rains begin early in June and last till the end of September, the total rainfall being about 27 inches per annum

In the cold weather the days are bright and warm, while the nights are cold and bracing The maximum temperature reached in December may be as low as 42°F

### WATER SUPPLY

The water supply is from Lake Fife, an artificial collection of water situated to the south-west of, and some 11½ miles by road, and 7 miles in a straight line from Poona city The water from this reservoir is carried by an open canal which runs roughly north-east of the vicinity of Poona city and then passes east through the cantonment The water in this canal is liable to pollution through the greater part of its course The supply for the city is taken from the canal at the south-west corner near Parvati and is chlorinated The cantonment supply is taken from the canal near St Mary's Church, passing through settling tanks and rapid filters, and is chlorinated

The daily bacteriological analysis of this supply throughout the year has shown a uniform absence of lactose fractors in 100 c c On 30 occasions, between the end of May and the beginning of November 1926, 500 c c were examined Lactose fractors were found to be absent from this quantity on all occasions except one (September 2nd)

season, but a rise in the number of cases commenced in June and increased in July and August, dropping in September

By the end of August 200 definite exudates, and 73 indefinite exudates had been bacteriologically examined. From the former *B. flexneri* was isolated in 61 per cent of cases, and *B. shiga* in 10 per cent of cases. From the latter exudates *B. flexneri* was isolated in 26 per cent of cases (17 *B. flexneri*, 2 *B. shiga*).

Clinically by far the great majority of the cases were extremely mild in character, the symptoms being usually those of a fairly acute diarrhoea, followed by the appearance of blood and mucus in the stools. In a comparatively large percentage faeces persisted in the stools throughout the attack, and blood and mucus were passed only in small quantities. This mild form of dysentery is typical of the condition known throughout the Cantonment as 'Poonaitis'. All grades of severity, however, were met with, up to really severe acute bacillary dysenteric attacks with high temperatures, severe tenesmus, and frequent stools consisting entirely of blood and mucus. The severity of the attacks appeared to depend very largely on the promptness with which proper treatment was instituted during the preliminary diarrhoea. Both *B. shiga* and *B. flexneri* were isolated from very mild cases, and very severe cases, i.e., the severity of the attack gave no indication of the nature of the causative organism. The *B. shiga* infections in 1925, were distinctly more severe than in 1926, when these cases reported sick as soon as diarrhoea commenced.

Cases from which *B. schmitzi* was isolated were distinctly severe in nature. Blood and mucus on the average persisted longer in the stools than in the shiga and flexner infections. The temperature, although not high, remained raised for several days, whereas in *shiga* and *flexneri* infections the temperature almost invariably dropped to normal after treatment by serum and salines had been instituted.

Careful records were kept by Captain W. Aitchison, M.C., R.M.S., on the special case sheets provided by the laboratory, of 150 patients.

In this series of cases, the average day after onset of symptoms on which blood was found microscopically to be present in the stools worked out at 4.6 days.

Mucus was found to persist up to the 5.45 day.

Faeces remained present in the stools throughout the illness in 32.5 per cent of cases.

In the more severe cases, in which the faeces had been replaced entirely by blood and mucus, faeces were reported as again being present in the stools on the following days after onset of symptoms —

2nd day	15.32	per cent of case			
3rd day	24.19				
4th day	29.03	"	"	"	"
5th day	14.51	"	"	"	"
6th day	12.9	"	"	"	"
7th day	3.22	"	"	"	"
9th day	0.8	"	"	"	"



Litter is now removed from the area by carts and motor vans and when not used in the filth pits is stacked in heaps, where, as noted some years ago by Lieutenant-Colonel Morison, I M S., apparently no fly breeding takes place

The writers would add to these notes —

- (i) That the Manick Nullah separates the city from the cantonment, and that this nullah is in practice merely a vast open latrine
- (ii) The open pans are emptied nominally by 10 a.m. and 6 p.m. and that during the remainder of the 24 hours the pan contents are exposed to the air
- (iii) Although latrines may be available at close range, such amenities are usually disregarded by the lower class Indian child and the ground beside the godown is generally selected in lieu
- (iv) Wherever building on any scale is proceeding, coolie camps spring up like mushrooms, and the sanitary arrangements of these often beggar description
- (v) The cantonment authorities permit officers and others to rent stables in their compounds to horse owners during the racing season, which corresponds to the dysentery season, and, as a result, 1,200 horses and their syces are scattered throughout the cantonment area, often under very unsuitable conditions
- (vi) During the season there are, as a rule, race days twice a week, and on these days thousands of Indians swarm into the cantonment from Poona city, and an extra strain is thrown on the existing sanitary arrangements

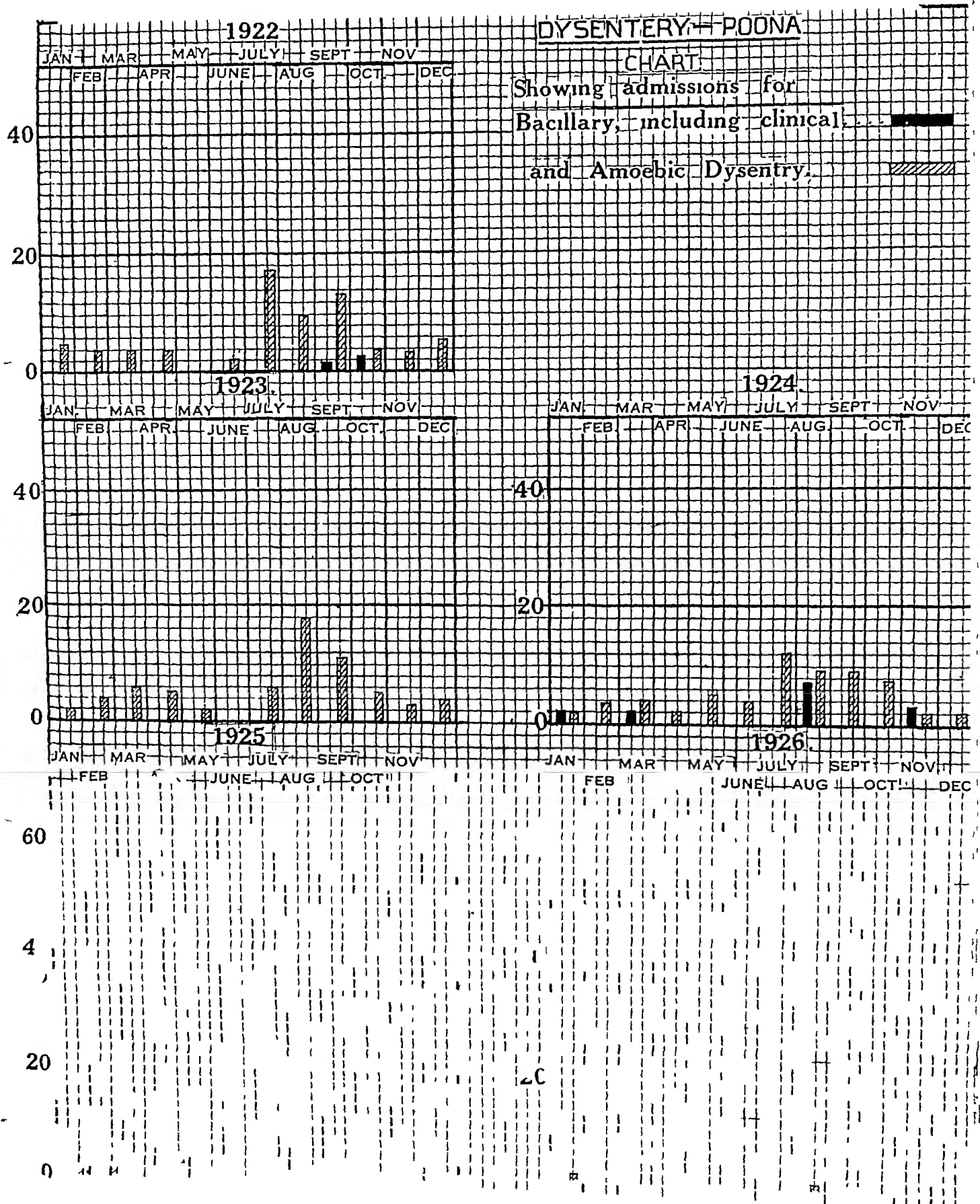
Bacillary dysentery and diarrhoea, as we have seen in Table V, are present throughout the year. There is a considerable rise in the number of cases in July and August, but there is no indication of any epidemic. Up to the end of July the average constantly sick for bacillary dysentery and 'dysentery group' for British troops was 2.84 per 1,000, and for Indian troops 1.88 per 1,000. These figures also represent numbers of cases who without specific orders would not have been diagnosed as such, and among the Indian troops particularly would not even have reported sick.

## METHOD BY WHICH INFECTION IS CONVEYED TO TROOPS

### WATER SUPPLY

Lieutenant-Colonel Morison working in 1912, 1913, traced very clearly the 'monsoon diarrhoeas' to the pollution of the water supply in Lake Fife. Daily records of the rainfall in 1926 are shown in Chart III for the months of June, July, August and September 1926. The monsoon broke on 8th June, the rain fell on the 10th, 11th, 13th and 27th of this month. Rain fell almost daily in July and August and from 12th to 17th September. We can trace no direct connection between the rainfall and the number of dysentery cases. In Chart III, it can be seen that the latter (considering that they are furnished from some thousands of individuals) show no evidence of any sudden increase after the heavy rainfall recorded on the chart. Chart IV shows that there is a comparatively steady

CHART I





isolated) confirmed our opinion that the majority of these cases were *flexner* infections. 57.4 per cent of these cases demonstrated a rise in their agglutinin content to *B. flexner* after their attack of dysentery. Serological examinations of cases of definite *flexner* infections showed that only 71.9 per cent produce an increase in agglutinin content to *B. flexner*.

None of the dysentery group cases developed an increased agglutinin content to *B. shiga*.

A detailed account of these serological examinations is given in Part II of this Report.

It will be seen from Chart I giving the hospital admissions for amoebic and bacillary dysentery (including 'dysentery group') for the past few years how large a number of dysentery cases must have been diagnosed under other headings in the past. The drop in the number of amoebic infections is also evident—and we would emphasise the fact that none of the 'dysentery group' cases have been treated with emetine during the years 1925-1926.

That similar conditions exist in other places than Poona is shown in Chart II giving the incidence of dysentery cases in Secunderabad for the same periods. Captain Loganadan, I.M.S., took up the investigation in the brigade laboratory there in the Autumn of 1924, with results similar to those we obtained in Poona. A rise during the monsoon period is also seen, and the predominant organism found is *B. flexner*.

Secunderabad is chosen as an illustration, as it has been considered in the past one of the worst stations in India as regards amoebic dysentery.

Similar conditions to those demonstrated in the above two stations have been found by other military laboratories also during 1925-26 in many other parts of India, both north and south, and that there is little doubt that India, as far as the army is concerned, will shortly fall into line with other eastern countries in this question of the differential diagnosis of the dysenteries.

#### SUMMARY OF CONCLUSIONS

1. Poonaitis is largely a mild *B. flexner* infection, and owing to its mildness has not been diagnosed as dysentery in the past.

2. Bacillary dysentery, again mainly *B. flexner* infection, accounts for most of the more severe cases of dysentery. These cases were usually diagnosed amoebic dysentery in former years.

3. *B. shiga* plays only a minor part in the production of dysentery in Poona, and if such cases report sick on the 1st or 2nd day of the attack and are treated at once, the cases are usually as mild as the *B. flexner* infections.

4. By the microscopic examination of mucus, it is possible to separate mild bacillary dysentery from diarrhoea in nearly all cases, if the hospitals and laboratory co-ordinate properly.

5. 'Dysentery group' as a diagnosis is a confession of failure, and in the main such cases in Poona have been *B. flexner* infections.

6. Bacillary dysentery and diarrhoea are present throughout all months of the year and increase in numbers in the monsoon period.

shower of rain, flies would swarm into buildings and high catches would be recorded unexpectedly. It was found best in the end to add all the records of catches together for each week and make a graph accordingly. The graph is shown on Chart IV, and on the whole bears a close relationship to similar observations in past years. The figures bear out also our own personal observations as to the prevalence of flies at particular periods. A glance at the graph will show that there is a very striking relationship between humidity, prevalence of flies, and incidence of bacillary dysentery. Also, it will be noticed that although the humidity remains high, the fly curve and number of dysentery cases drop in September. This was also apparent in 1925 and the same drop in the number of flies was noticed by Lieutenant-Colonel Morison in 1913. In all probability it is as he states due to a fungus such as *Empusa muscæ* found in England. Time was not available to study this matter, but it is a subject which should be investigated. Temperature, humidity, and all other conditions for fly breeding remain, but the numbers of flies invariably drop at this period of the year.

The relationship between the number of flies caught and the number of dysentery admissions is even closer than indicated in the chart.

The units returning the fly counts divided each month into four periods, and sent in numbers caught as per week. The admission of dysentery cases were worked out for periods of 7 days irrespective of the day of the week. Hence in the diagram the peak of the fly curve is shown after the peak of the dysentery curve, actually they coincided. The actual findings are shown below —

Week ending	Dysentery admissions, July and August		Number of flies caught, July and August.	
	2nd July	8 cases	1st to 11th July	1,340
" "	9th July	10 cases	11th to 18th July	1,578
" "	16th July	10 cases	18th to 25th July	4,050
" "	23rd July	15 cases	25th to 31st July	4,939
" "	30th July	16 cases	31st July to 8th Aug	6,232
" "	6th August	18 cases	8th to 15th Aug.	2,803
" "	13th August	16 cases	15th to 22nd Aug	2,810
" "	20th August	15 cases	22nd to 29th Aug	1,855
" "	22nd August	14 cases		

It is again emphasised, however, that fly counts can only be taken as indicating within broad limits the prevalence of flies in the periods indicated. That flies were most prevalent between the 18th of July to about the 14th August was undoubted.

Chart IV also shows well the number of fresh cases met with apart from the monsoon incidence. On the whole the number remains fairly constant, with occasional rises and falls, i.e., the sudden drop in the week ending 3rd September. This latter was not due to cessation of rain, as rain had fallen each day from 22nd to 31st August. We have, therefore, the fact that bacillary dysentery cases, diarrhoea cases, and flies are present in Poona throughout the year and largely rise and fall together. The water supply from strict bacteriological tests, and from the evidence of the relationship of the rainfall to cases, appears to have no direct relationship, under the existing conditions of chlorination, to these diseases.

## SANITATION

The disposal of filth and refuse in the Poona area is still extremely unsatisfactory, although with the provision of a water carriage system, which is now under construction as a joint scheme, there is some hope of improving it

Latrines are practically all of open types. In some parts of the area, e.g., the cantonment, attempts have been made to form public latrine groups. While such grouping is of value in ensuring better supervision it undoubtedly leads to fouling of the ground in areas away from these groups. This is specially marked along the city side of the Manick Nullah where there are very few latrines.

In addition to the latrine groups there are many isolated latrines of private ownership, or situated in the compounds of private bungalows.

Disposal of night-soil is on the removal system and each latrine or latrine group is equipped with covered receptacles to which the pan contents are transferred pending transference to the filth carts, or as in Poona city, to the pail depôts of the small water carriage system at present serving with that area.

The transfer of the pan contents to the receptacle in which they are protected from flies takes place in the public latrines nominally twice daily. In the private latrines much less attention is as a rule paid to this and the pan contents may remain exposed for considerable periods. In all cases the continued exposure is obviously a dangerous procedure.

From the latrines the covered receptacles are taken and emptied into the filth carts for removal to the trenching ground, except as noted above for Poona city.

There are two trenching grounds for the area. That of the suburban municipality is situated close to the river bank and just to the north of the recently developed Koregaon Estate. It should here be noted that pan contents from a part of the suburban municipal area situated to the left bank of the river are carried back to the trenching ground which is now close to a residential area.

The cantonment trenching ground is situated some 1,800 yds to the east of the cantonment boundary.

The pits of these trenching grounds are filled with a mixture of pan contents and litter, or dry refuse, which is allowed to rot and is then dug out and sold as manure.

The soil is unsuitable for trenching and a very large amount of fly breeding takes place in the pits especially in the period August to October. Close observation during the past few years has shown that few flies from these pits travel into the inhabited area.

The methods of collection of stable litter are unsatisfactory. Practically no Manure Pits exist and anything like complete removal is exceptional, especially from the end of May to the end of October when there is an addition to the annual population of some 1,200 horses assembled for the Poona racing season. Under these conditions fly breeding from this source is exceptionally heavy.

CHART V

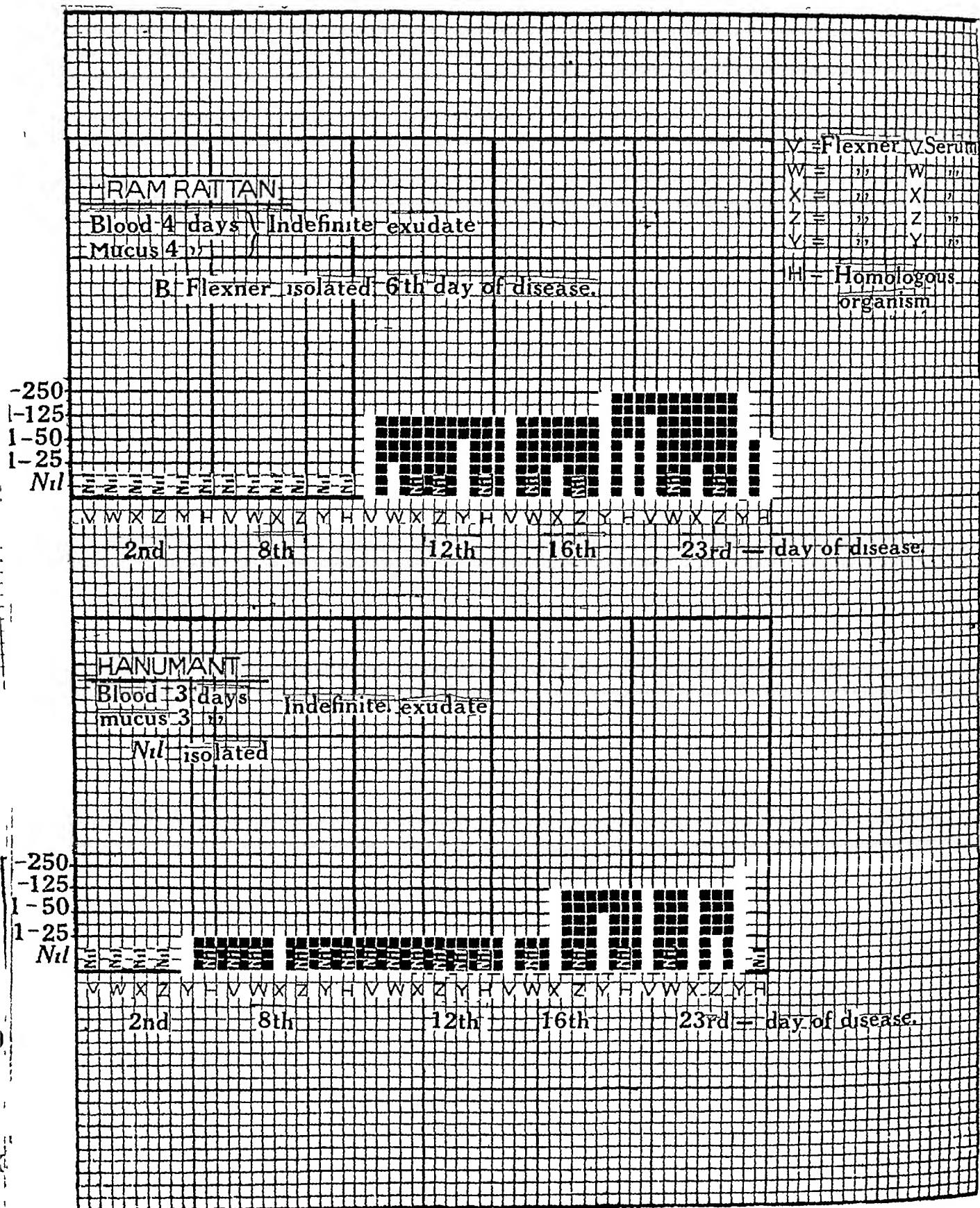
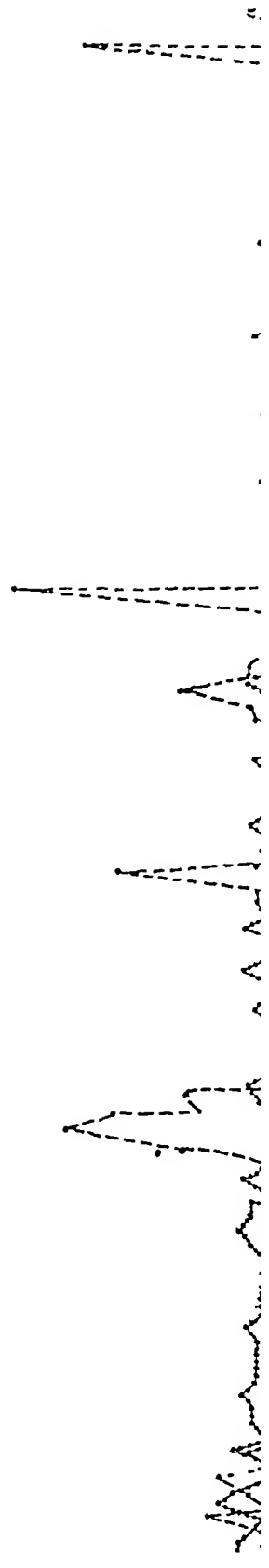
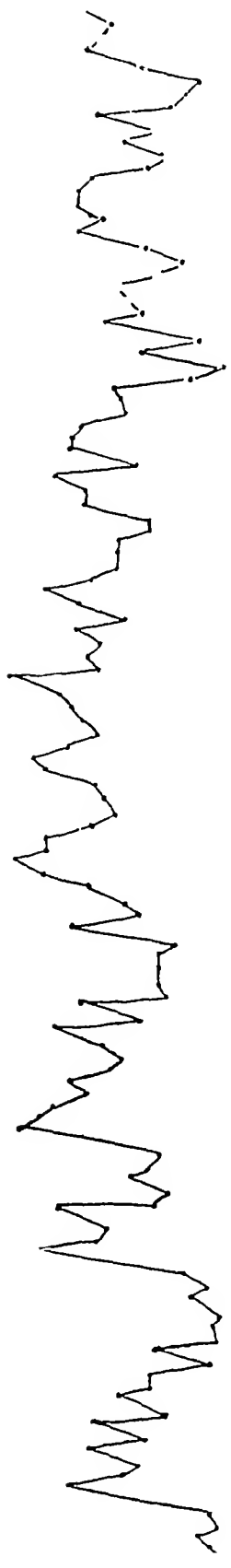


CHART III

JUNE	JULY	AUGUST	SEPTEMBER
123456789101112131415161718192021222324252627282930	123456789101112131415161718192021222324252627282930	123456789101112131415161718192021222324252627282930	123456789101112131415161718192021222324252627282930





we provided, and that the laboratory should send daily to collect these from the cantonment office. The specimens were to be labelled with the number of the latrine from which they were collected.

*June*—This series of examinations commenced on 13th June. During the period 13th to 30th June, 52 specimens of blood and mucus arrived in the laboratory. These were examined microscopically. 26 were definite bacillary dysentery, 17 were reported as indefinite but suggestive of bacillary dysentery. In addition 30 other specimens of loose stools in which the sweepers stated blood and mucus were present, were received, but as no blood and mucus could be seen in the specimens they were not bacteriologically examined owing to pressure of routine work. From the 52 blood and mucus specimens 10 *B. flexner* and 1 *B. shiga* were isolated.

*July*—120 specimens were collected in which the sweepers stated blood and mucus were present. 57 contained sufficient mucus for examination and 1 was a large intestinal slough. 29 were typical bacillary exudates and 28 indefinite. *B. flexner* was isolated in 11 cases.

After July no further specimens were received, and as we considered we had obtained sufficient evidence of the presence of infective material supplied by the local population in the cantonment and had more work in the laboratory than could be dealt with, we did not press the matter.

Specimens had been received from over a large area in the cantonment and in considerable numbers. If it is realised that the specimens were collected by ignorant sweepers without any skilled supervision, the numbers actually received must have only been a fraction of those existing. Many of the specimens must have been lying for hours before they reached the laboratory. *B. flexner*, as was expected, was the dysentery organism isolated in greatest numbers.

It appears to us very evident that latent bacillary dysentery is as great a danger in Poona as in Madras, and that there is an ample supply of *Bacillus flexner* available for the fly to pass on.

Further evidence of the extent of the *flexner* or *shiga* infections among the troops, we thought might be gained from the serological examinations of individuals not suffering from dysentery, or from dysentery cases in the early stages, i.e., 1st or 2nd day, before agglutinins caused by the attack of dysentery could have developed.

We commenced therefore in January examining as many individuals as possible on those lines. The blood serum was put up against the five standard *B. flexner* strains, and *B. shiga* standard Oxford cultures. The lowest dilution of serum used was 1 to 25.

The results in brief were that 31.8 per cent British troops (average service in India, 2 to 5 years) and 50.6 per cent Indian troops contained in their serum agglutinins to one or all of the standard *flexner* strains. *Flexner V* appeared to be the predominant strain among both British and Indian troops.

Actual details of titres found, etc., are given in the laboratory notes at the end of this report.

incidence of dysentery throughout the monsoon months, high from weeks ending 23rd July to 27th August. Heavy rain had commenced and continued daily from 5th July, but during the weeks ending the 9th and 16th July there were only two cases admitted per week more than in the week 21st May in which month rain had fallen on the 3rd and 4th days only. These two weeks in July show fewer cases admitted than in the week ending 19th February, a month in which there was no rain at all. Rain also fell from 12th to 18th September following on a period of ten days dry weather and the drop in the dysentery admissions continued. Similarly in the month of June there is no particular increase in the number of cases. In fact the number of diarrhoeas recorded was considerably fewer than in May, when except for small showers there was no rain, i e.,

	Dysentery	Diarrhoea
May	28	24
June	34	14

The chlorinated water is, as Major Wallace states, examined bacteriologically daily. Lactose fermenters throughout the year have been absent in 100 c c, and during the monsoon period in 39 tests were only found once in 500 c c. We have also frequently tested the tap water in the laboratory and have never found lactose fermenters in 100 c c. This is as high a standard of purity as could be obtained in any water supply.

That dysentery organisms are easily destroyed by other bacilli and die out soon after passage in the stools, is a commonplace, and therefore, that these organisms should remain alive in the water of Lake Fife for four days (minimum) and still persist in water which is sufficiently chlorinated to destroy resistant lactose fermenting organisms in 500 c c seems to us to be impossible.

Further there is no evidence of other water-borne diseases among the troops in the cantonment. Any enteric cases have been traced to carriers except for one or two isolated cases, and there have been no cases of cholera.

Whatever the conditions may have been in the past, it appears quite obvious to us that the water supply is not now concerned with the spread of either diarrhoea or dysentery in Poona, and that the rainfall, except indirectly, by producing other factors, has no connection with the dysentery and diarrhoea increase in the monsoon.

### FLIES

Fly traps were distributed by Major Wallace among the various units, and placed in cookhouses, coffee bars, institutes, etc. A record of the weekly catch was asked for from the Medical Officer in charge of the unit.

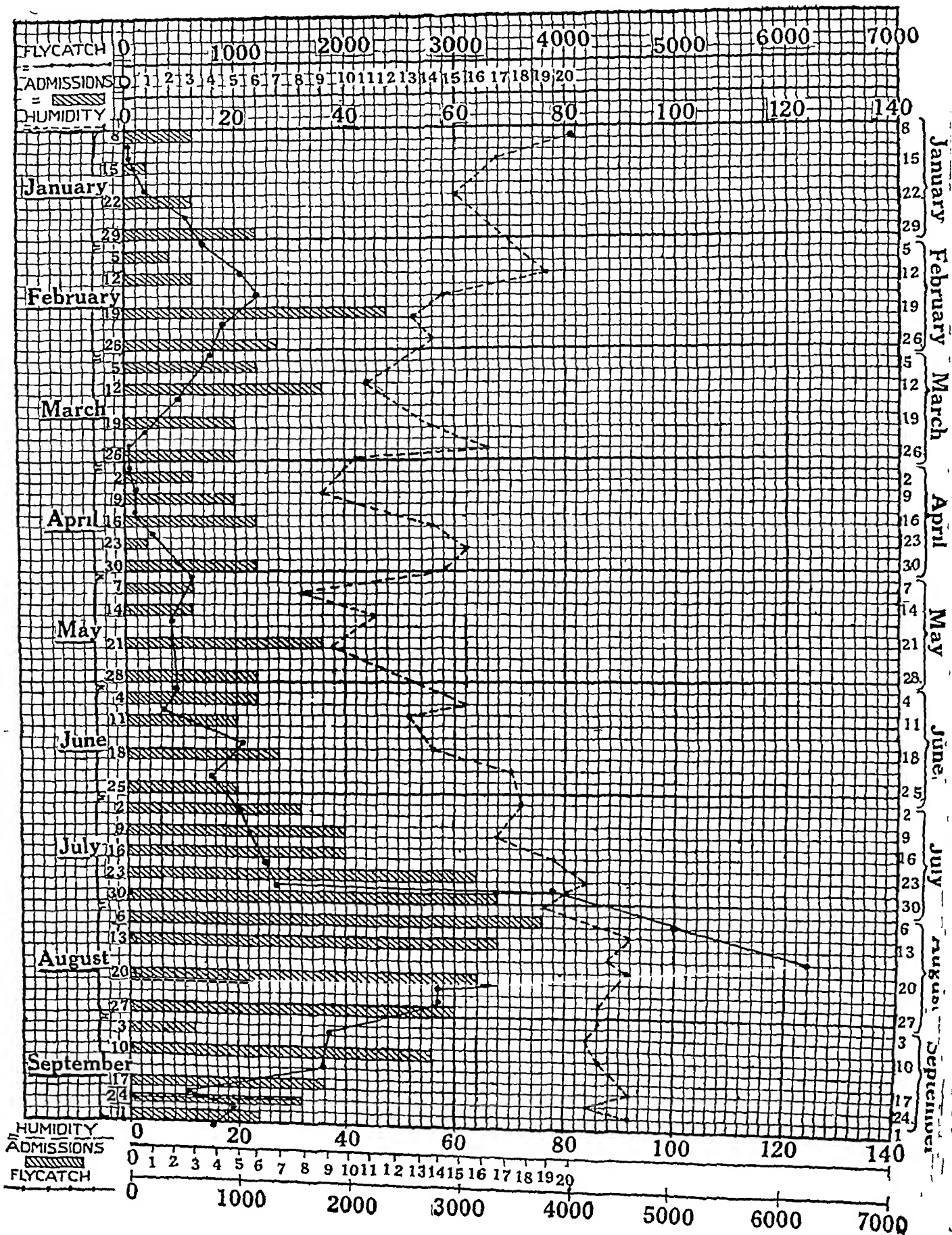
It was very soon found that the figures obtained would only give a very broad indication of the number of flies prevailing. For example, some Medical Officers were considerably more active than others in supervising the baiting of the traps and the actual counting and recording of the flies. The habits of flies were found to be extremely variable. Many units used fly-papers in addition to the traps and often these were covered with flies, while the fly-traps remained empty. There would appear often to be few flies about, and after a sudden

## REFERENCES

(References are not given to the extensive standard literature of bacillary dysentery, but only to those consulted for the particular purpose of this report, or being of particular local interest)

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- (4) ACROB and KNOWLES (1924) *Ind Med Gaz*, LIX, Nos 7 and 9
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CHART IV.



A dry plate is of course absolutely essential. Plating out on a wet plate is merely a waste of time.

On the next day likely colonies were picked off, if possible five from each plate, and inoculated direct into glucose and mannite peptone salt solution, plus Andrade's indicator. After 24 hours' incubation of these sugars, it was possible to run through the series of tubes quickly, and discard any organisms not fermenting either glucose and mannite, or glucose only, without gas. This method saved a great deal of time, and in practice was very efficient. Colonies giving the reactions of *flexneri* group, or of *B. shiga*, were then dealt with by subculturing from the glucose tube into lactose, dulcitol, peptone salt and broth. The lactose tube, if the sugar was not fermented in 24 hours, was kept in the incubator for three weeks to ascertain if the strain was a late lactose fermenter. The peptone salt culture was tested for indol by Dr. Gore's technique using Bohme's solutions. The broth culture was first examined by the hanging drop method for motility, and then formalinized by the addition of formalin to 1 per cent and kept for serological tests.

Teague and Clurman's glycerin and saline solution was found to be very efficient, when specimens had to be sent from outstations, and *B. flexner* was isolated frequently in specimens sent from Aurangabad, Belgaum and Ahmadnagar by train. Dried films of mucus from outstations were also found useful as a means of diagnosing the bacillary exudate. We found the best stain for these to be hæmatoxylin, and Biebrich's scarlet as a counter stain, after fixation in methyl alcohol. As a matter of fact if a reasonably large sample of mucus was placed in the glycerin and saline solution and not emulsified too thoroughly, this solution preserved the cells wonderfully well. In cases of suspected amœbic dysentery coverslips from outstations sent in 70 per cent alcohol after fixation in Schaudinn's solution were examined after staining by Heidenheim's iron-hæmatoxylin (long method).

*Macroscopic appearances* of the stools were in the main as described by Acton, Knowles and other authors. In the mild *flexner* cases, particularly among Indian troops, the mucus was often in very small amounts and was frequently missed by the hospital subordinate whose duty it was to select the sample for transmission to the laboratory. In bacillary cases it was invariably neutral or alkaline to litmus paper.

*Microscopic appearances* appeared to us more important than the macroscopic, and the diagnostic value of the cellular exudate simply cannot be overstated. In acute cases two seconds' examination after a little experience are sufficient for diagnostic purposes.

In the very mild *flexner* cases, however, careful search was often necessary to find the small collections of polymorphonuclear leucocytes among the debris of the loose stool. Had we been able to select the sample for examination ourselves from the entire stool there would probably have been less difficulty. Such specimens were often reported upon as indefinite but suggestive of bacillary dysentery.

The results of bacteriological examinations of these cases have already been given, and the results of serological tests on the patients are discussed later.

The next step was to ascertain whether by bacteriological examinations the local flies could be proved to be flies of flexner bacilli

Owing to pressure of work not many examinations of flies were carried out, but the results of the few that were done appeared to prove conclusively, that not only are flies capable of acting as carriers, but that the fly infection is a heavy one. During August flies were sent to the laboratory on 8 occasions from the coffee bar of the 2nd battalion K S L I. The method of examination was carried out on lines sent to me by Dr Manson-Bahr, i.e., twelve flies after a thorough flaming were emulsified in peptone salt solution and the supernatant fluid plated out.

On three occasions out of the eight, organisms biochemically and morphologically identical with *B flexner* were isolated. On the first occasion this organism was in pure culture on the plate. One of the three organisms isolated was agglutinated to a titre of 1-50 by flexner W and Z serum (test carried out by Dreyer's method). In September two further *B flexner* were isolated in ten examinations. Further work is being carried out on similar lines by Major Walker, R A M C, now in Poona, and he informs me that he has already isolated 2 further *B flexner* agglutinating with polyvalent serum.

In addition all the various other faecal organisms encountered in our bacteriological examinations of dysentery cases were isolated in these few fly examinations, including, among others, *B morgan* No 1 on several occasions.

The case against the fly appears to be proved, and indeed this finding agrees with experience in many other parts of India.

In places in which the conditions of the water supply, rainfall, etc., are quite different from one another—for example Quetta and Secunderabad—bacillary dysentery (flexner) comes to a head in the fly season. Dr Manson-Bahr showed some years ago that similar conditions prevailed in Fiji. Throughout the east two common factors in the production of intestinal disease can always be found, i.e., flies and faeces on which flies can feed, however much other conditions may vary.

#### SOURCE OF ORIGIN OF *B flexner*

The next step was to find the source of origin of the supply of *B flexner* to the troops. Lieutenant-Colonel Cunningham's work had shown that latent dysentery in Madras and Bengal was extremely common. If similar conditions prevailed in Poona, the open pan latrine with its contents exposed for hours, and possibly days, to the fly population appeared to be the most probable source of infection. Civil figures from hospitals and dispensaries could not help us much, as little or no attempt has been made to separate bacillary from amoebic dysentery. The gaol population of Yarowda was well outside cantonments, and therefore could not be a source of infection. Attention was therefore directed to the cantonment public and servants' latrines.

Lieutenant-Colonel Reid, the cantonment executive officer, kindly gave us access to the sanitary inspectors, who arranged that the sweepers should collect any specimens of blood and mucus they found into faeces collection tubes, which

# SHANKER MANU

typical Bacillary exudate

Shiga isolated 2nd

ay of disase.

1-250

1-125

1-50

1-25

Nil

1st 12th 6th

Day

MOTRIS

ndefinite xu lat

B. Shiga isolated

2nd ay of disase.

1-250

1-125

1-50

1-25

Nil

typical Bacillary exudate

B. Shiga isolated

3rd day of disase

1-250

1-125

1-50

1-25

Nil

2nd 8th 12th 16th 20th

Day

2nd 8th 12th 16th 20th

ay of disase

1-125

1-50

1-25

Nil

1-250

1-125

1-50

1-25

Nil

2nd 8th 12th 16th 20th

Day

2nd 8th 12th 16th 20th

ay of disase

Among the 187 cases examined, on only three occasions were agglutinins to *B. shiga* standard culture found present in dilutions of 1 to 25 or over. One, a European of many years' service in India, had been complaining for some time of abdominal discomfort with occasional passage of mucus. Unfortunately he refused to have any bacteriological examinations carried out. The other two were Indians. Both had suffered from frequent attacks of dysentery, and in both cases *B. shiga* was isolated.

In view of these findings over a large number of examinations, it would appear worth while to consider the question of carrying out serological examinations in order to detect carriers of *B. shiga*. We expected to find a large proportion showing agglutinins in 1 to 25 or over, but except as noted above, none were found.

Possibly the use of other agglutination methods than Dreyer's may account for the accepted idea that agglutinins to *B. shiga* are present in a large number of healthy individuals in the tropics in a dilution of serum over 1 to 25.

That 'contact infections' occurred apart from the agency of flies, appeared to us to be proved by the frequency with which the 'mothers' of children suffering from dysentery and treated in quarters, were infected while nursing their children. Many such cases occurred among families including those of officers. If such cases can occur among individuals who understand the dangers of infection, and who are scrupulously clean, we can easily realise the ease with which the Indian servant passes on his infection. The fact that a little mucus is passed is too trivial an occurrence to merit any particular attention as regards increased cleanliness of the hands in these individuals.

#### SUMMARY AND CONCLUSIONS

1 Chlorination of the water supply in Poona is efficient. The water supply is not responsible for either dysentery or diarrhoea now endemic in the cantonment.

2 There is a heavy infection among the Indian population in the cantonment of bacillary dysentery (*B. flexner*).

3 This is passed by them to the European population and to each other by direct contact (hands, etc.), throughout the year.

4 Flies are responsible for the large increase in these diseases during the monsoon period.

5 The existing sanitary arrangements in Poona are absolutely ideal for the spread of bacillary dysentery.

In conclusion we have to thank Major G. Wallace, O.B.E., R.A.M.C., for his notes on the sanitation of Poona, and for arranging and supervising the fly counts, etc., Major Dowse, M.C., R.A.M.C., Major Pottinger, M.C., R.A.M.C., and Captain Aitchison, M.C., I.M.S., for giving us the benefit of their clinical observations, and also for the trouble they have taken to ensure that specimens from their cases were sent to the laboratory in such a manner as to give some chance of a positive isolation of the infective bacillus.



*Organisms other than dysentery bacilli encountered in the bacteriological examinations of bacillary dysentery stools*

At the commencement of this investigation we recorded these other intestinal bacilli, but it was soon obvious that if the hospitals and the laboratory were absolutely up to the mark as regards co-ordination, these other bacilli were only of secondary or of no importance

In the later stages of the disease organisms of the *B. faecalis alkaligenes* group were almost universally present and *B. morgan* No 1 was found in the great majority of cases sometimes early along with *B. flexner*, but more often from the 3rd day onwards. In some cases where we had failed to find *B. flexner* but large numbers of *B. morgan* No 1 had been present daily, we endeavoured to find evidence of agglutinins in the patient's serum to this bacillus, but were never successful in dilutions of serum of 1—25 or over

Another bacillus was frequently met with fermenting glucose only, but without gas. This bacillus, although really motile, was often non-motile when first isolated. As it produced indol it might possibly be confused with *B. schmitz* unless subcultured several times, and examined for motility each time. An agglutination test with *B. schmitz* serum of course settled any doubt. We considered this bacillus to be a strain of the *morgan* group. Several patients' serum was also tested against this bacillus without result.

*B. schmitz* was encountered on several occasions and serologically proved. The agglutination results of 11 tests with the patients' serum are given later under serological tests.

Late lactose fermenting organisms in all respects resembling *B. flexner* were isolated in a certain number of cases. It was found necessary to keep the lactose tubes of apparent *B. flexner* for long periods in the incubator to collect these.

Some strains were found to be turning the indicator as late as the 17th day. In all 19 strains were isolated. All were tested by Michaelis acid agglutination test and three gave flocculation. The remainder showed no flocculation. Three of the latter have agglutinated to a reasonable titre by monovalent *flexner* serum, i.e., 75 per cent in eight hours, two further strains in low titre. Owing to lack of time we have not completed the investigation of these strains, but hope to do so later. Similar bacilli have never been encountered by us apart from an attack of dysentery during the numerous stool examinations carried out in the past two years.

A coliform organism fermenting glucose and mannite with acid and gas, but not dulcitate, and fermenting lactose late (5th day onwards) was frequently encountered in the late stages of the disease, and in chronic mild cases with few symptoms but abdominal discomfort and occasional passage of microscopically indefinite mucus. In one such case a vaccine prepared from this bacillus was immediately successful in clearing up the condition.

Mixed infections of amœbic and bacillary dysentery we found present in only 2 per cent of our cases. In these there was present a typical bacillary exudate, and *B. flexner* was isolated, but the condition did not clear up, and eventually *E. histolytica* was found and the case responded at once to emetine.

# REPORT ON AN INVESTIGATION OF DYSENTERY AND DIARRHŒA IN POONA

## Part II.

### LABORATORY FINDINGS

BY

MAJOR J A MANIFOLD, D S O , R A M C ,

ASSISTED BY

ASSISTANT SURGEON A J DeMONTE, I M D

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### I BACTERIOLOGICAL EXAMINATIONS

BLOOD and mucus from 190 bacillary cases in 1925, and from 300 up to the end of September 1926, were examined in the district laboratory

These figures refer only to cases among the troops in Poona and Kirkee, and are exclusive of specimens sent in from outstations

#### TECHNIQUE

The routine carried out was that a portion of mucus was picked up on a platinum loop, rinsed gently in sterile saline, and then streaked across a bile salt litmus lactose agar plate by means of the platinum loop

At the same time another specimen of mucus was placed on a slide, flattened out under a coverslip and examined with the 1/6 lens

It is important that only a small portion of mucus be taken for this examination in order that as thin a specimen as possible be examined The result of microscopic examination was then sent to the M O 1/c case 'by the bearer of specimen,' stating either that a bacillary exudate was present, *E. histolytica* present, or that no definite opinion could be given This advance report was found very useful from a treatment point of view

The time at which the stool was passed, and of its plating out, plus the reaction of the mucus and the macroscopic and microscopic appearance were entered in a special register

Litmus lactose bile salt medium was found much the easiest to work with, once good litmus granules were obtained

101 complete serological tests were carried out on the above class of cases

Definite exudates 79 cases (47 *B flexner* isolated)

Indefinite exudates 22 cases (7 *B flexner* isolated)

#### SEROLOGICAL EXAMINATIONS OF THE 79 DEFINITE CASES

55 (69.6 per cent) show a rise in titre to one or all strains of *B flexner*. 22 of the 55 had nil agglutinins present when first examined. Agglutinins were found present for the first time in most of these cases on the 12th day, and on the 12th day the rise in titre for the already existing agglutinins became apparent.

The maximum titres reached against the standard Oxford strains by these 55 sera are as follows —

	SERUM OF 55 PATIENTS				
	versus				
	Flexner V	Flexner W	Flexner X	Flexner Z	Flexner Y
Nil agglutinins present throughout test	4	7	17	24	As flexner V but
Titre 1—25		13	12	7	in rather less
Titre 1—50	10	10	16	10	proportion
Titre 1—125	19	8	9	7	
Titre 1—250	22	7	1	7	
	—	—	—	—	
Total were tested	55	55	55	55	

#### DAY OF DISEASE ON WHICH MAXIMUM TITRE WAS FOUND PRESENT AMONG THE 55 CASES

4 cases (7.2 per cent) reached highest titre on 8th day of disease

12 „ (21.7 per cent) „ „ „ „ 12th „ „ „

11 „ (20 per cent) „ „ „ „ 16th „ „ „

28 „ (52.7 per cent) „ „ „ „ 23rd „ „ „

From the above figures it will be seen that agglutinins to *flexner V* predominated in these patients' sera, being absent in only four cases out of 55, and also that the 55 sera agglutinated *V* in a higher titre on the average than the other strains.

In very few instances did the agglutination reactions to the various strains give sufficient indication of the type of infecting strain of *B flexner* as will be seen from the histograms picked at random from those made on these 55 tests. Occasionally one strain did predominate, but owing to the irregular appearance of the agglutinins in general, little value could be attached to this.

#### *B flexner* CASES

From both definite and indefinite exudates 54 *B flexner* were isolated. 39 of these cases gave a rise in titre to the standard *flexner* cultures, i.e. 71.9 per cent. Of the 15 showing no rise, 8 cases had an entire absence of

# CHART VI

PRASAD LAL

Blood 2 days  
mucus 3 days

Definite Bacillary exudate

B. Flexner isolated 2nd and 3rd day

V = Flexner  
W = Serum  
X =  
Z =  
Y =  
H = Homologous organism

1-250  
1-125  
1-50  
1-25  
Nil

V W X Z Y H V W X Z Y H V W X Z Y H V W X Z Y H  
2nd 8th 12th 16th 23rd = Day of disease

DILAWAR KHAN

Blood 8 days  
mucus 2 days

Definite Bacillary exudate

B. Flexner isolated 2nd day of disease

1-250  
1-125  
1-50  
1-25  
Nil

V W X Z Y H V W X Z Y H V W X Z Y H V W X Z Y H  
2nd 8th 12th 16th 23rd = Day of disease

MEHER KHAN

Blood 4 days  
mucus 5 "   
Nil isolated

Indefinite exudate

1-250  
1-125  
1-50  
1-25  
Nil

V W X Z Y H V W X Z Y H V W X Z Y H V W X Z Y H  
2nd 8th 12th 16th 23rd = Day of disease

## SUMMARY OF CONCLUSIONS ON SERIES OF TESTS

- 1 71.9 per cent *B flexner* infections produced agglutinins to *B flexner*
- 2 57.4 per cent 'group' infections demonstrated a similar production of agglutinins to *B flexner*
- 3 Agglutinins to *B flexner* appeared irregularly usually about 12th day, and in most cases were highest on 23rd day after onset
- 4 *Flexner V* agglutinins appeared to predominate but that this can be taken as an indication that *flexner V* is the main infectious strain is doubtful
- 5 The severity of attack bore little relation to the production of agglutinins
- 6 *B shiga* agglutinins were not found to be present in any of the sera of the dysentery group cases tested

## III SEROLOGICAL EXAMINATIONS

Serological examinations of British and Indian troops not suffering from dysentery, or on 1st or 2nd day after onset of dysentery, before agglutinin due to existing attack could become apparent in the serum

	British troops—66	Indian troops—121
	British Troops	Indian Troops
	Per cent	Per cent
Percentage showing standard agglutination to any strain of <i>flexner</i> in a titre of 1—25 or over	31.8	50.0
Percentage showing agglutinins to —		
<i>Flexner V</i>	66	73.9
<i>Flexner W</i>	23.8	26.2
<i>Flexner X</i>	38.1	42.7
<i>Flexner Z</i>	28.6	27.9
<i>Flexner Y</i>	42.9	54.0

The actual titres of agglutination reached against the different strains were as follows —

British Troops	1—25 Per cent	1—50 Per cent	1—125 Per cent	1—250 Per cent	Per cent
<i>Flexner V</i>	18	35	13	=	66
<i>Flexner W</i>	14.3	9.5		=	23.8
<i>Flexner X</i>	14.3	14.3	9.5	=	38.1
<i>Flexner Z</i>	14.3	14.3		=	28.6
<i>Flexner Y</i>	4.8	23.8	14.3	=	42.9
Indian Troops					
<i>Flexner V</i>	24.6	23	23	3.3 =	73.9
<i>Flexner W</i>	13.1	18.2	4.9	=	26.2
<i>Flexner X</i>	16.4	19.7	4.9	1.7 =	42.7
<i>Flexner Z</i>	14.8	11.4	1.7	=	27.9
<i>Flexner Y</i>	11.4	21.3	18	3.3 =	54.0

*Importance of speed in despatch of specimens to the laboratory*

The time at which the stool was passed was entered upon all laboratory reports sent with the specimen to the laboratory, and was on the whole fairly accurate. At any rate the fact that the time had to be entered impressed on subordinates that speed in despatch was important. On looking through our records the greatest number of *B. flexner* were isolated from fresh specimens received in the laboratory two hours after passage, and even up to three hours, but not beyond this period except in very few cases, unless the specimen had been sent in the 30 per cent glycerin in 6 per cent saline solution. From specimens received in the laboratory under 30 minutes after passage a positive result was almost invariable.

*Importance of sending specimens to the laboratory early in the disease*

Our records show clearly the importance of specimens being sent to the laboratory as early as possible in the disease.

Positive results were obtained as a rule on 1st, 2nd and 3rd day.

In 100 *flexner* infections the bacillus was isolated up to the 4th day in 13 instances —

5th day	in	11	instances
6th	"	7	"
7th	"	4	"
8th	"	3	"
12th	"	1	instance
14th	"	1	"
15th	"	1	"

In only six cases in this series was *B. flexner* isolated in the absence of mucus, once on the 5th day, four times on the sixth day, and once on 14th day after onset of the disease.

The isolations on 12th and 15th day were from cases in which blood and mucus had been absent for a few days but had recurred.

In view of the fact that throughout all our examinations we have found *B. flexner* present in normal stools without mucus on such few occasions, the routine examinations of the faeces of *flexner* cases once the stools are formed appear hardly worth the time and expense. If arrangements could be made for the patient's stool to be examined macroscopically on eight consecutive days after discharge from hospital for the presence of mucus, the results would probably be equally good, if not better than bacteriological examinations, as regards the detection of convalescent carriers.

The above comments as regards the importance of speed in despatch of specimens to the laboratory, and the sending of specimens early in the disease apply equally if not more so to cases of *B. shiga* infections. Our results appeared to show that most *shiga* infections can be rendered as harmless as *flexner* infections if dealt with on correct lines in the very earliest stages of the disease.

other observers. In two other cases serum taken from cases on the 2nd day of the illness agglutinated both the homologous organism and the Oxford standard culture of *B. shiga* to a titre of 1—125. Both had a history of previous attacks of dysentery. Four of the cases showed nil agglutinins either to homologous organisms or to the standard culture of *B. shiga* in a titre of 1—25. The severity of the attack appears to have had no effect on the formation of agglutinins. Indeed the most severe attack in the series (raised temperature for 12 days, blood and mucus persisting for 19 days on and off) showed no evidence of agglutinin response. The patient in addition was suffering from *Tamiasaginata* infection. The majority of the cases were extremely mild in nature. This as has been remarked before was probably due to the fact that patients reported sick on the first or 2nd day of the disease and were promptly treated.

#### SEROLOGICAL TESTS ON CASES FROM WHICH *B. schmitz* WAS ISOLATED

Complete tests were carried out on 11 cases. Four of these cases produced agglutinins against the laboratory culture of *B. schmitz*. The extent and period of agglutination are shown in the histograms (Chart VIII).

The homologous organism was in no case agglutinated by the patients' serum. As the stock culture was agglutinated in four cases this result is probably due to inagglutinability of recently isolated cultures. It should be noted that the organism was agglutinated to a reasonable titre, i.e., 1—250 by laboratory serum 1—500. Presumably the agglutinins in the patient's serum were not sufficiently developed to overcome the inagglutinability of the bacillus.

In view of the fact that *B. schmitz* as an agent in the production of dysentery has been considered by some observers to be doubtful owing to the non-production of agglutinins in the patient's serum, the production of agglutinins in these few cases appears worth recording.

#### V SEROLOGICAL OBSERVATIONS ON STRAINS OF *B. FLEXNER* ISOLATED

During the routine serological examinations of *B. flexner*, the inagglutinability of freshly isolated strains had been marked.

Some after several subcultures became agglutinable, but a considerable number still remained after many months entirely inagglutinable to a polyvalent *flexner* serum of high titre.

Owing to this inagglutinability it was thought best to collect a fair number of strains, and keep them in the hope that as time passed they would become more agglutinable.

In all 117 strains were put on one side for this purpose from cases occurring in 1925, and in the early months of 1926. We attempted to type these in August and September 1926. The results have been interesting, but on the whole not satisfactory, and for that reason are considered worth recording.

We took the Medical Research Committee Special Report Series No. 42 as a guide, and endeavoured to follow out as far as possible the technique which Professor Andrews and Dr. Inman had found satisfactory. In the introduction to this report it is stated that from 1916 onwards, 'It was found everywhere

In one case we isolated from the same specimen of faeces *B flexner* and *B shiga*. Both were agglutinated by their specific serum, and by the patient's serum to a titre of 1—250 *B shiga*, 1—125 *B flexner*. Presumably the patient was a carrier of one organism, and had received a recent infection of the other.

## II SEROLOGICAL EXAMINATIONS OF PATIENTS SUFFERING FROM BACILLARY DYSENTERY (INCLUDING GROUP)

According to the existing Regulations for the Medical Services in India a case cannot be diagnosed as bacillary dysentery unless an organism of the dysentery group is actually isolated. If a bacillary exudate is noted, in the absence of the infective organism the official diagnosis is 'dysentery group (bacillary exudate)'. If no bacillary exudate is reported, the diagnosis remains as dysentery group. Our experience has shown that the presence or absence of a bacillary exudate and the numbers of positive isolations depend on many factors, many of which are outside the control of the laboratory, and that the greater percentage of these 'group' infections are really bacillary in origin. Therefore as many cases as possible were tested serologically from all types 'Bacillary dysentery,' 'dysentery group' (definite exudates or indefinite exudates).

Serum was obtained from patients on the first or second day of the disease, and on the 8th, 12th, 16th and 23rd day from onset of symptoms.

1 Serum of cases from whom *B flexner* was isolated was put up against standard Oxford cultures of *B flexner* V, W, X, Z, Y, and against the homologous organism on each occasion, i.e., five times over a period of 23 days.

2 Serum of cases from whom no infective organism was isolated was put up in a similar manner against the five *flexner* stains, and also against a standard culture of *B shiga* both from cases showing definite and indefinite exudates.

3 On the first test every patient's serum was put up against all the strains of *B flexner* and *B shiga*.

4 Serum of cases from whom *B shiga* was isolated was put up against *B shiga* standard culture, and against the homologous organism, at the same intervals of time.

5 Serum of cases from whom *B schmitz* was isolated was put up against a formalinized 24-hour broth culture of the stock laboratory *B schmitz* and against the homologous organism five times in 23 days.

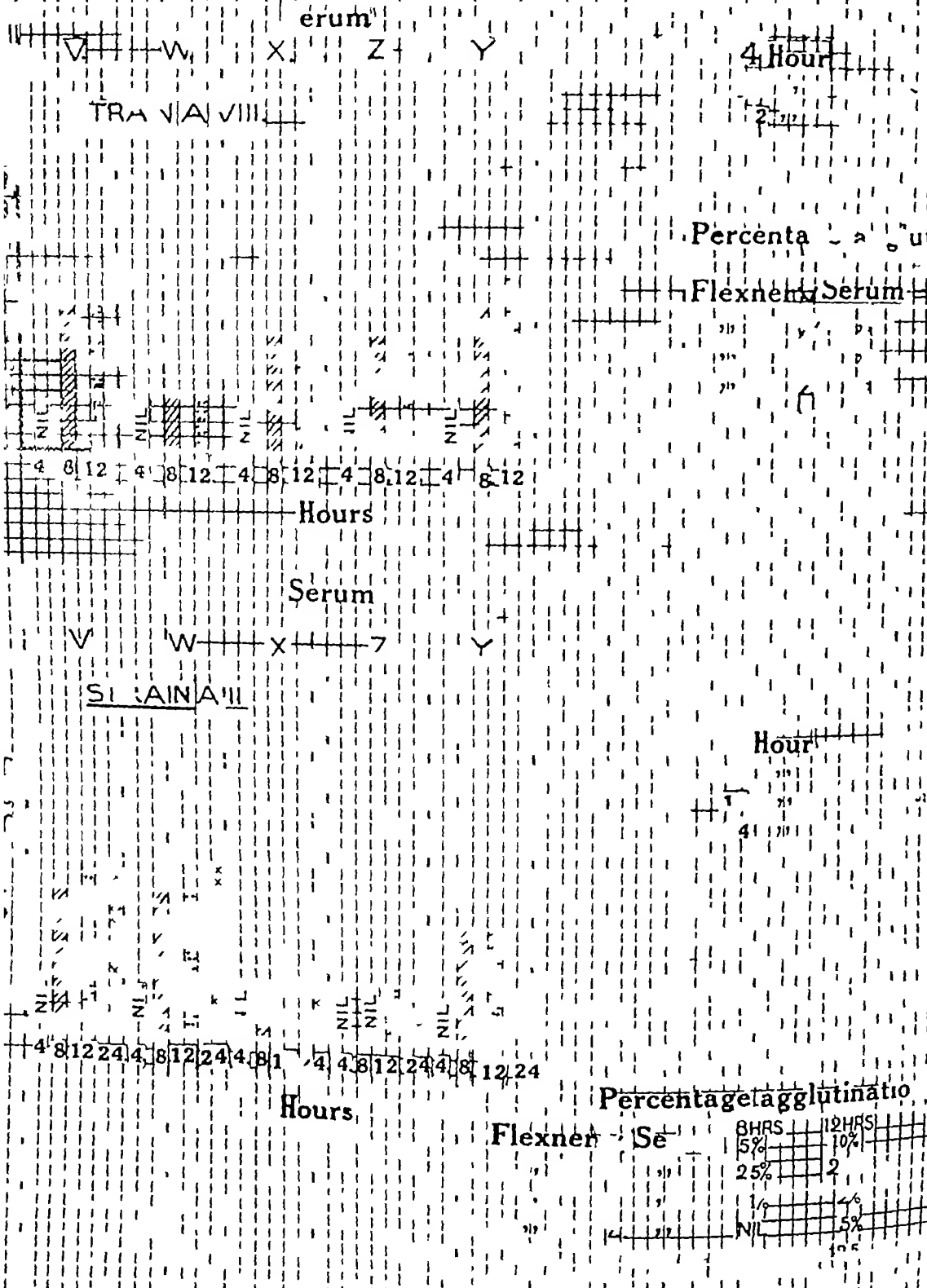
6 The homologous organism used was always a 24-hour formalinized broth culture. In all cases Dreyer's agglutination technique was used, and the Dreyer's tubes were allowed to remain in the water bath at 55°C for four hours.

### SEROLOGICAL TESTS ON CASES FROM WHICH *B flexner* WAS ISOLATED AND ON DYSENTERY GROUP CASES

Throughout the tests 'nil agglutinins present' indicates that no agglutinins were demonstrable in a dilution of serum of 1—25, the lowest dilution employed throughout.



Demonstrating change in titre of agglutination  
after different periods of time in water bath at 55°C



agglutinins to *B flexner* throughout the test. The remainder had agglutinins present when first examined, but these either remained at the original titre or dropped subsequently.

The severity of the cases appears to have had no connection whatever with the formation of agglutinins. Many of the mildest cases have given good agglutination results, and more protracted cases have failed to produce agglutinins. The following case may be taken as an example.

Pte T 20-1-26. Blood and mucus 4 days—*B flexner* isolated, and agglutinated 1—250 by polyvalent dysentery serum. Complete tests. Nil agglutinins to any strain of *B flexner* including homologous organism.

4-7-26. Blood and mucus 5 days (sharp attack) *B flexner* isolated and agglutinated by polyvalent *flexner* serum 1—250. A complete series of tests again showed nil agglutinins except that on the 16th day the serum agglutinated *flexner* X to 1—50. On the 23rd day nil agglutinins were again present. The organism isolated was put up against all five monovalent *flexner* sera, and was agglutinated to 50 per cent titre by *flexner* W serum, and to 2.5 per cent titre by *flexner* X serum. The patient's serum did not agglutinate this bacillus throughout.

The homologous organism was agglutinated by the patient's serum in only 22 instances (40.7 per cent). The histograms show that in these instances the patient's serum became as a rule capable of agglutinating his own bacillus late in the disease, i.e., on 16th to 23rd day, occasionally in low titre as early as the 12th day. The organism in many instances was agglutinated by polyvalent laboratory serum, but not by the patient's serum, and, as will be seen later, many were inagglutinable either by the patient's serum or by laboratory serum.

If 8 hours had been allowed for agglutination to occur before reading the results it is probably in view of our experience later, that a higher titre of agglutination might have been found with the patient's serum, and possibly a larger number of the organisms would have been agglutinated.

#### SEROLOGICAL RESULTS ON 47 CASES WITH INDISTINCT EXUDATES (7 *B flexner* ISOLATED)

11 of the 22 (50 per cent) showed a definite rise in titre in all respects similar to those discussed above.

From the 11 cases showing no rise, 2 *B flexner* were isolated.

Dysentery Group Cases, i.e., no organisms isolated.

The serological test on these 47 cases demonstrated that 57.4 per cent gave a definite rise in agglutinins to *B flexner*.

28.1 per cent cases from which *B flexner* was actually isolated show nil agglutinins to any strain of *B flexner*. It appears therefore to us that as was suggested by clinical symptoms most of these group cases are mild *flexner* infections.

In none of the 101 cases did any agglutinins to *B shiga* become apparent in the patient's serum throughout the period of examination (23 days).

as nearly as possible equal to that of an Oxford laboratory standard culture. The titres of *flexner V*, *W*, *X* and *Y* serum were tested on similar cultures of their homologous organism for each batch of broth made up. The dilution of serum in which each strain tested showed standard agglutination, was expressed as a percentage of the titre of the serum to its own homologous bacillus.

Dreyer's method was used throughout, the tubes being left for four hours in the water bath at 55°C, the readings then being taken by artificial light against a black background.

The lowest dilution of serum employed was 1—25, and the highest 1—5,000. The titre of the monovalent serum used varied a little from time to time, but usually the *flexner V* serum gave a titre of 1—5,000, and *flexner W*, *X*, *Z* and *Y* 1—1,000. Occasionally one or other of the latter was higher in titre.

#### RESULTS OF THE 4-HOUR READINGS

Of the 117 cultures 51 or 43·5 per cent failed to show any evidence of agglutination with *V*, *W*, *X*, *Z* and *Y* serum in 1—25 dilution, and many of those, which did agglutinate, did so only in low dilutions. Before attempting to type according to these results we repeated a large number of the tests but left the tubes in the water baths up to 24 hours, readings being taken at 4, 8, 12 and 24 hours. We found that the readings were nearly always appreciably higher at 8 than at 4 hours, but in only a few instances was the reading at 12 or 24 hours higher than the 8-hour readings.

Also cultures definitely inagglutinable (nil in 1—25) at four hours, were often definitely agglutinable by 8 hours to reasonable titre. Inhibition zones were extremely constant in most strains often up to 1—125, and 8 hours in the water bath appeared to overcome this to some extent.

As an example the titre of some of the stock *flexner* laboratory serum tested at one period may be given.

	4-hours' reading	8-hours' reading	12-hours' reading,
<i>Flexner V</i>	1—2,500	1—5,000	1—5,000 (Standard+)
<i>Flexner W</i>	1—500	1—1,000	1—1,000
<i>Flexner X</i>	1—500	1—2,500	1—2,500
<i>Flexner Z</i>	1—500	1—500	1—500
<i>Flexner Y</i>	1—500	1—1,000	1—1,000

It will be seen that there is a marked difference between the 4 and 8-hour readings, particularly in *flexner X* serum.

The increase in titre is shown in histograms, i.e., strain A viii, A 11 showed no agglutination in four hours but definite agglutination in eight hours. Strains A ix, A 17, show the great increase in the titre found at the 8-hour reading.

The increase in titre did not appear necessarily to alter the grouping of the strain, as is seen in histograms for strain A ii presumably a *flexner W*. But in some cases it did apparently affect the typing, i.e., strain A ix at the 4-hour reading was agglutinated by *X* serum to 25 per cent titre, and by *V* serum to only 1 per cent, whereas at the 8-hour reading it was agglutinated by *V* serum to 50

From the above figures it is evident that *fleiner V* agglutinins are present in a larger percentage, and to a higher titre than the agglutinins of the other *fleiner* strains. *Fleiner Y* appears to be the next in frequency, but as at a late stage in these investigations the standard *fleiner Y* culture appeared to have become auto-agglutinable, it is felt that the *fleiner Y* results may not be as accurate as in the case of the other strains used. Although no actual sedimentation was seen in the control tubes, the emulsions may have been unduly sensitive to the action of the serum. *Fleiner W* was found present in a smaller percentage and in lower titre than *fleiner X*, or *Z*. Higher titres were found to be present in a larger proportion of Indian troops than amongst British troops. In no case was standard agglutination present in a higher dilution than 1—250, and as high as 1—250 in only a small percentage of cases. Sera from all the 187 cases were also tested against a standard culture of *B. shiga*. To our surprise standard agglutination in a titre of 1—25 or over was only found present on three occasions. One, a European of many years' service in India, had been complaining for some time of abdominal discomfort, with occasional passage of mucus. Unfortunately he refused to have any bacteriological examinations carried out. The other two cases were Indians. Both had suffered from frequent attacks of dysentery, and in both cases *B. shiga* was isolated.

In view of these results it would appear worth while to consider the question of carrying out serological examinations using Dreyer's method, and Oxford standard cultures, to detect carriers of *Bacillus shiga*. We had expected to find large numbers particularly among the Indian troops demonstrating agglutinins to this bacillus in as low a titre as 1—25. The small proportion of infections with *B. shiga* among the acute dysentery cases, and the fact that none of the 'dysentery group cases' examined developed agglutinins to this bacillus appear also to support the supposition that *B. shiga* infections take only a small part in the normal incidence of dysentery in Poona.

#### IV SEROLOGICAL OBSERVATIONS ON CASES OF *B. SHIGA* INFECTIONS

A complete series of tests were carried out on 12 cases only. That further tests were not made is due to the fact that infections with this bacillus are uncommon (22 from January 1st to September 30th) and that some of these infections occurred among women and children from whom no serum was obtained.

8 of the cases demonstrated the presence of agglutinins to *B. shiga* by the 12th day, i.e., 66.6 per cent. These patients' serum also agglutinated the homologous organism on either the 12th or 16th day. With the exception of two cases the maximum titre was reached on the 23rd day. The maximum titre varied from 1—125 to 1—250.

The percentage of cases demonstrating the presence of agglutinins was smaller than we expected. But the number of observations is small, and if further cases had been available the percentage showing the formation of agglutinins would probably have been higher, as has been found to be the case by

TABLE VI

Comparison of agglutinins in patients' serum and percentage of titre of agglutination of the organisms isolated from these cases against flexner V, W, X, Z and Y

Agglutinins in Patients' serum			Percentage of agglutination of the organism isolated with sera of V, W, X, Z, Y		
1	Flexner IV strain— 1 Jit Singh 23rd day	Flexner V 1—250	Per cent		
			1	in Flexner V serum	
		Flexner V 1—250	100	„ „	W „
		„ W 1—125	2 5	„ „	X „
		„ X 1—125	12 5	„ „	Z „
		„ Z 1—50	25	„ „	Y „
		„ Y 1—250			
	Homologous bacillus	1—250			
2	Abdulla Bag 16th day	Flexner V 1—50	2 5	„ „	V „
		„ W Nil	100	„ „	W „
		„ X 1—25	5	„ „	X „
		„ Z Nil	12 5	„ „	Z „
		„ Y 1—50	25	„ „	Y „
3	Walters 16th day	Flexner V 1—250	10	„ „	V „
		„ W 1—50	100	„ „	W „
		„ X 1—25	37 5	„ „	X „
		„ Z 1—25	Nil		Z „
		„ Y Control ?	25	„ „	Y „
	Homologous bacillus	1—50			
4	Tickaram 9th day	Flexner V 1—50	1	„ „	V „
		„ W 1—125	50	„ „	W „
		„ X 1—50	2 5	„ „	X „
		„ Z 1—125	Nil	„ „	Z „
		„ Y 1—250	5	„ „	Y „
	Homologous bacillus	1—125			
5	Lall Khan 23rd day	Flexner V Nil	10	„ „	V „
		„ W 1—125	100	„ „	W „
		„ X 1—125	5	„ „	X „
		„ Z	5	„ „	Z „
		„ Y ? ?	25	„ „	Y „
	Homologous bacillus	1—250			

# CHART VIII.

PATIENTS' SERUM V B SCHMITZ STOCK LABORATORY CULTURE

BHAWALI

250  
125  
70  
25  
Nil

2nd 8th 12th 6th 23rd  
a of disea a

KANAYALAL

25  
12  
50  
25  
Nil

2nd 4th 12th 16th 23rd  
Day of disease

ALLJ

250  
125  
50  
25  
Nil

2nd 8th 12th 16th 23rd  
Day of disease

HAD

2  
12  
5  
Nil

2nd 8th 12th 16th 23rd  
Day of disease

TABLE VI—concl'd

Agglutinins in Patients' Serum				Percentage of agglutination of the organism isolated with sera in V, W, X, Z, Y			
				Per cent			
<i>Flexner Y strain—</i>				Nil	in <i>Flexner</i>	V serum	
1	Mitchell	23rd day	<i>Flexner V</i> 1—250	5	„	„	W „
			„ W 1—25	25	„	„	X „
			„ X 1—125	12.5	„	„	Z „
			„ Z 1—50	50	„	„	Y „
		Homologous bacillus	„ Y 1—125				
2	Yates	23rd day	„ V 1—250	7.5	„	„	V „
			„ W 1—25	37.5	„	„	W „
			„ X 1—50	100	„	„	X „
			„ Z 1—125	50	„	„	Z „
			„ Y 1—250	200	„	„	Y „
		Homologous bacillus					

agglutinins were greatly predominating, the percentage of agglutination titre to the V serum was uniformly low in almost all the strain tests

Looking up the records of agglutination tests on patients during and after the dysenteric attack, it was found possible in a few cases to compare the patients' agglutinins, with the percentage titre of agglutination of the homologous organism against the five standard sera

*Table VI*—Gives the details in five cases from which apparently *flexner W* was isolated, 2 cases of *flexner X* infections, 3 cases of *flexner Z* infections, and 2 cases of *flexner Y* infections

If the typing of strains by the above method is correct the agglutinins produced in the patients' serum correspond in very few cases with the strain of the infecting organism, and as will be seen V agglutinins appeared to predominate in the patients' serum in nearly every case irrespective of the strain of the infective bacillus

*The agglutminogenesis of strains* were tested in 2 cases picked out at random —

- (1) In one of these, Ram Rattan, we had records of the test on the patient's serum. From the Chart XI it will be seen that this strain by the agglutination method would have been classified as a probable *flexner Z* (Z 50 per cent, X 20 per cent, V and Y

that organisms assignable by culturable and chemical characteristics to the *flexner* group failed to respond to the final test by agglutination.

Strains were therefore collected by these workers from different parts of the world, and were ultimately assigned to five main strains, and two sub-strains, i.e., *V*, *Z*, and *W*, *X*.

The majority of the known *flexner* strains were included in these groups, and by the use of specific monovalent serum, it was considered that nearly all the *flexner* groups could be assigned to their proper strain.

The methods used in grouping were—

- (a) Agglutination tests
- (b) Agglutininogenesis
- (c) Absorption tests

The agglutination tests were carried out first, and the results were confirmed agglutininogenesis experiments with the various strains and finally by the absorption test.

The results of the agglutination test were demonstrated by plotting out the effect of each serum upon the bacillus under investigation, expressing the result as a percentage of the full titre of the serum with its homologous bacillus. It was then possible to show 'at a glance' the strain to which the bacillus belonged. It was also found by Professor Andrews and Dr. Inman that '*flexner* bacillus with some exceptions agglutinates with fair readiness, flocculation being nearly complete in 4 to 6 hours at 55°C. In certain *flexner* strains a delay in agglutination was occasionally noted, but on the whole the difference between the early and late agglutinating species is so striking that it may be regarded as of special significance.' The readings throughout their tests were accordingly taken after 4 hours in a water bath at 55°C.

The agglutination method of typing strains was obviously that most suited to workers such as ourselves in a district laboratory, in which routine work is heavy, and takes up most of the available time. It would be impossible to carry out either agglutininogenesis or absorption tests on the large numbers of *flexner* bacilli isolated. Our attention was accordingly directed mainly to the agglutination method, which appeared simple and applicable to any district laboratory.

The 117 strains tested by us were morphologically and biochemically *B. flexner*. All were isolated from dysentery cases, and as a rule on 2nd, 3rd day of disease. All were gram-negative non-motile bacilli, fermenting glucose and mannite without the production of gas, and not fermenting lactose up to three weeks in the incubator. Some strains produced indol. Others did not. In addition all were tested by Michaelis acid agglutination test and none gave any evidence of flocculation at the end of two hours. Some late lactose fermenting strains not included in this series, did give coarse flocculation by this test.

#### METHOD EMPLOYED

Formalin (1 per cent) was added to a 24-hour broth culture (Lab. Lemco) of the organism to be tested. The formalinized cultures were left in the ice chest overnight, and next morning were diluted with normal saline to an opacity



# CHART XI

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per cent titre and by X only to 10 per cent (titre of X serum 1—500 at four hours, and 1—2,500 in eight hours)

It appears to us, therefore, that it is safer to take readings at 8 hours in the water bath, when endeavouring to place *flexner* bacilli in their various categories by the agglutination method, and also when testing by polyvalent or monovalent serum to prove their identity as *B flexner*. Very many bacteriologists prefer to take their agglutination readings with *B flexner* at 24 hours for similar reasons, but owing to the results given in Medical Research Report Series No 42 teaching, we understand, has in late years inclined to be that 4-hour readings are sufficient. Such is not our experience.

As a result of the above findings we decided to start again, and once more all the 117 strains were put up against all five monovalent *flexner* serum, and the readings were taken after 8 hours in the water bath.

#### RESULTS OF THE 8-HOUR READINGS ON 117 STRAINS

1 33 (28.2 per cent) strains were still inagglutinable (1—25), i.e., 15.3 per cent less than that at the 4-hour reading.

2 Of the 84 which showed agglutination —

(a) 60 strains (51.2 per cent) appeared to have one sufficiently predominant antigen demonstrated to enable them to be placed in a definite category.

(b) 24 strains (20.5 per cent) although agglutinating, showed only a small percentage of titre of agglutination, with the various sera, or else agglutinated to such a high titre and to similar percentages with several sera, that an absorption test would obviously have been necessary to settle the question of their identity.

The 60 strains fell into the following groups —

21 (35 per cent) = *Flexner W*

11 (18.3 per cent) = *Flexner Y*

10 (16.6 per cent) = *Flexner Z*

9 (15 per cent) = *Flexner X*

7 (11.6 per cent) = *Flexner V*

2 (3.3 per cent) = *Flexner V, Z*

*Flexner W* being the predominant strain, and *flexner V* the least frequent.

As *flexner V* agglutinins predominated in the tests on the serum of patients suffering from dysentery, and also in the sera of normal individuals, the above results, if correct, were distinctly unexpected. The above test relies on the agglutinating power of the serum to overcome the inagglutinability of the organism tested to the same extent with all the sera used, i.e., the serum of the same strain as the bacillus tested should agglutinate it to the highest percentage, and the remaining sera should agglutinate it in diminishing proportions. The agglutinability of *B flexner* strains has appeared to us to be such an erratic factor, that we feel uncertain that this is the case in reality, except possibly with stock laboratory cultures. Except in the 7 strains noted in which *flexner V*,

cultures It was put up against 29 other strains (14 inagglutinable and 15 indefinite), and agglutinated 6 of these heavily in six hours, four further strains in 12 hours, and the majority of the remainder showed faint granulation in 1—25 or 1—50 at 24 hours This granulation did not appear to us to resemble true agglutination but was more in the nature of a fine precipitation, and this appearance seems to us an argument against taking readings at such a late stage As one of the writers was transferred from the laboratory, almost directly after these tests were done, further work on this bacillus had to stop for the time But it appears that we are dealing here with a strain of *B flexner* which bears little or no relationship to the five standard strains One of the assistant surgeons attending a course in the laboratory has been found to have agglutinins present to this strain in a titre of 1—125

Probably among these definitely inagglutinable bacilli there are others not included among the recognised strains

Although incomplete we think these results worth recording, as several D A D Ps, not only in India but in other parts of the world, have been finding that polyvalent *flexner* serum or monovalent *flexner* sera made from the five standard strains of *B flexner*, have not yet solved the problem of the inagglutinability of the *flexner* strains

In time organisms undoubtedly become more agglutinable In fact an Oxford laboratory standard *flexner* Y culture had become auto-agglutinable We have kept strains now well over a year and still some remain inagglutinable The final serological proof of the identity of a bacillus as *B flexner* must therefore from a practical laboratory point of view be absent in many instances One cannot wait two or three years before giving a final diagnosis The bacteriologist must therefore still depend mainly on the morphology, cultural and biochemical characters of these organisms for his diagnosis apart from serological proof We personally consider that these are sufficient from a practical point of view

Final serological proof can only be carried out on all strains when some method of reducing the inagglutinability is found We endeavoured to do this by growing strains in broth to which various percentages of glucose had been added, but in every case the resulting cultures were definitely auto-agglutinable

It is regretted that the serological investigations had suddenly to cease owing to the unexpected transfer of the writer to duties outside a laboratory It was intended to make sera from all the apparently *flexner* W strains, and carry out agglutination and absorption tests in every case, our feeling being that many of these strains might prove to be other strains of *B flexner* than *flexner* W We had hoped to examine carefully the group of inagglutinable cultures, which were agglutinable with serum of strain Finch, and if possible to investigate similarly the other inagglutinable cultures The relationship of the few lactose fermenting bacilli, which agglutinated with *flexner* serum, to *B sonnei* on the one hand and *B flexner* on the other, would also have been investigated The enormous number of *flexner* strains available in the military laboratories in India supply a large field for study, and it is hoped that other Deputy Assistant Directors of Pathology will complete this work in the near future

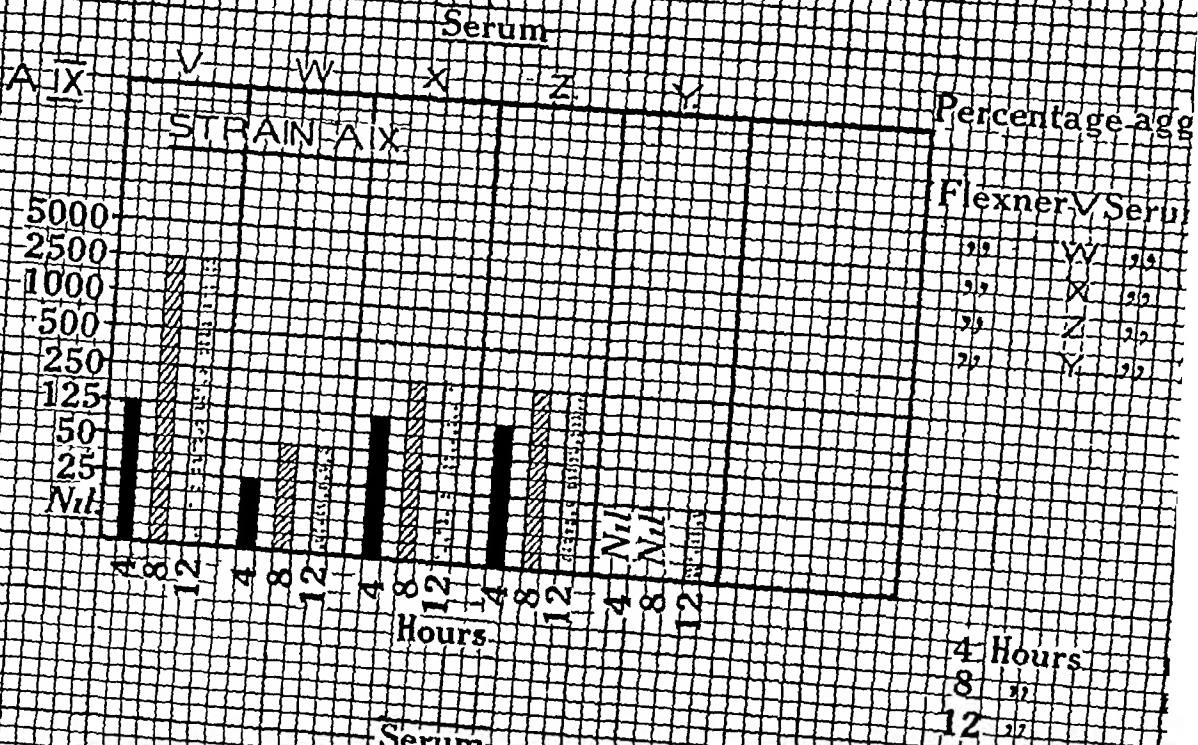
TABLE VI—contd

Agglutinins in Patients' Serum				Percentage of agglutination of the organism isolated with sera of V, W, X, Z, Y			
<i>Flexner X strain—</i>				Per cent			
1	Fateh Mohd	16th day	<i>Flexner V</i> Nil	16	in <i>Flexner V</i> strain		
			" W 1—125	50	"	"	W "
			" X 1—50	200	"	"	X "
			" Z Nil	75	"	"	Z "
			" Y ? ?	100	"	"	Y "
2	Oakley	16th day	" V 1—87 5	3	"	"	V "
			" W 1—37 5	25	"	"	W "
			" X 1—25	100	"	"	X "
			" Z Nil	50	"	"	Z "
			" Y ? ?	50	"	"	Y "
<i>Flexner Z strain—</i>							
	Ram Rattan	23rd day	<i>Flexner V.</i> 1—25	1	in <i>Flexner V</i> serum.		
			" W Nil	Nil			
			" X. 1—25	20	"	"	X "
			" Z .	100	"	"	Z "
			" Y 1—125	2 5	"	"	Y "
	Homologous bacillus .		1—50				
2	Supati Manu	16th day	" V 1—125	1	"	"	V "
			" W Nil		"	"	W "
			" X Nil	10	"	"	X "
			" Z Nil	50	"	"	Z "
			" Y 1—50	5	"	"	Y "
	Homologous bacillus.		1—50				
3	Mardan Shah	23rd day	" V 1—250	Nil	"	"	V "
			" W 1—25	Nil	"	"	W "
			" X 1—25	Nil	"	"	X "
			" Z 1—25	50	"	"	Z "
			" Y 1—125	Nil	"	"	Y "
	Homologous bacillus		" 1—125				



CHART X

Demonstrating change in titre of agglutination  
after different periods of time in water bath at 55°C



Our first line of investigation then was to obtain a standard based on a study of the local population With this end in view we examined the cholesterol content of the whole blood in one hundred normal cases

The results of this investigation are given in Table I

TABLE I  
*Cholesterol in Whole Blood (Normal)*

Case No	Initials	Cholesterol content per cent	REMARKS
1	S G	0 17	Hindu male, age 62
2	S B	0 14	Ditto, age 14
3	M	0 13	Ditto, age 12
4	J N S	0 114	Ditto, age 24
5	P C	0 138	Ditto
6	Y H	0 11	Md male, age 25
7	B M	0 106	Hindu male, age 25.
8	M L	0 084	Ditto
9	D	0 123	Ditto
10	Sk M B	0 116	Md male
11	K M	0 133	Hindu male
12	G C D	0 114	Ditto
13	K Sk	0 184	Md male
14	M I	0 167	Ditto
15	T C	0 092	Hindu male
16	Sk I	0 09	Md male
17	E H	0 104	Ditto
18	S D	0 114	Hindu male
19	C D	0 092	Ditto
20	H K C	0 126	Ditto
21	G K	0 092	Ditto
22	B	0 12	Ditto
23	S M	0 123	Ditto
24	D S	0 105	Ditto
25	B	0 10	Ditto
26	S M	0 096	Ditto

1 per cent) whereas the rabbit serum prepared from this bacillus agglutinates *flexner V* to full titre, and *flexner Z* hardly at all. The organism in question being undoubtedly a *flexner V*. The agglutinins produced in the patient's serum agree with the latter finding.

- (2) Strain Gridhar Lal demonstrates well the inagglutinability of some of the cultures (this strain was isolated early in 1925). From the agglutination test it could not be placed in any strain owing to the low percentage of agglutination (*V* 1 per cent, *W* 2.5 per cent, *Y* 5 per cent) apparently the organism is a *flexner Y*. This strain was inagglutinable in four hours.

In at least one instance therefore, in which the agglutination method was checked by the agglutino-genesis the former appears to have given an incorrect reading. A 50 per cent titre is possibly not much to go upon, but the homologous serum in this case only agglutinated the bacillus to 1 per cent titre, whereas a heterologous serum (*Z*) agglutinated it to 50 per cent titre.

#### INAGGLUTINABLE CULTURES

Four strains were picked at random from amongst the 33 absolutely inagglutinable bacilli in order to test the agglutino-genesis of these organisms.

In Research Report Series No. 42 Professor Andrews and Dr. Inman state that a single dose of  $\frac{1}{2}$ —1 c.c. formalinized broth culture given intravenously commonly yields a serum of titre 1—1,000 in a week, and that a repetition of the above suffices to raise the titre to 1—3,000 or more.

This also in the past had been our experience while working with stock laboratory cultures of *B. flexner*, except that  $\frac{1}{4}$ ,  $\frac{1}{2}$  and 1 c.c. doses were given at four-day intervals. No difficulty had been obtained in producing a serum of high titre.

Our experience with these inagglutinable strains, however, has been entirely different, and in only two of the four cases could a serum of reasonable titre be obtained, and then after considerable trouble. One strain produced an agglutinating serum with comparative ease, and turned out to be a *flexner Z*.

With the other strains live cultures, in doses of  $\frac{1}{2}$  and 1 c.c. were eventually employed, after numerous doses of dead cultures had been given, with the result that several rabbits were killed. A serum of high titre was eventually obtained with only one of these strains (strain Finch). With the other 2 strains with a serum with titre higher than 1—250 could not be obtained, and as a result they were useless for our purpose.

Apparently inagglutinability and weak agglutino-genesis correspond in many instances among the *flexner* strains. Strain Finch had been isolated in the early months of 1925, and had remained entirely inagglutinable to *flexner V*, *W*, *X*, *Z* and *Y* serum until September 1926, when a trace of agglutination was evident in a dilution of 1—50 *W* serum. The serum (titre 1—5,000) prepared from this strain did not agglutinate either *V*, *W*, *X*, *Z* or *Y* Oxford laboratory standard.



TABLE I—(contd)

Case No	Initials	Cholesterol content per cent	REMARKS
58	A S	0 114	Md
59	N J	0 12	Ditto
60	G N	0 126	Hindu
61	S A. M.	0 109	Md male
62	J S.	0 114	.. .
63	M	0 133	.. .
64	N. D	0 12	Hindu male
65	B	0 123	Ditto
66	S Ch N	0 114	Ditto
67	G K A.	0 096	Ditto
68	P	0 082	Ditto
69	K	0 114	Ditto
70	M. I	0 10	Md
71	M. R	0 12	Ditto
72	J	0 096	
73	B	0 114	Hindu
74	S K D	0 184	Ditto
75	M K K	0 11	Md.
76	U	0 145	
77	U K	0 106	Md
78	T	0 156	Ditto
79	S D	0 16	Hindu
80	R K S	0 109	Ditto
81	R D	0 12	Ditto
82	R N	0 114	Ditto
83	N U O	0 109	Ditto
84	M	0 109	Ditto
85	R	0 109	Ditto
86	M M	0 11	Ditto
87	S S D	0 17	Ditto
88	K	0 104	Ditto

SUMMARY OF RESULTS

- 1 Of 117 strains 71.7 per cent only are agglutinable by *flexner* V, W, X, Z and Y serum after many months' subculturing
- 2 Four hours in the water bath at 55°C are insufficient to overcome inhibition zones, and inagglutinability. Eight-hour readings have given higher titres of agglutination, and reduced the number of inagglutinable cultures by 15.3 per cent
3. Only 51 per cent of the agglutinable cultures examined could be assigned to definite strains by the agglutination method given in Medical Research Series Special Report No. 42

The results did not correspond with those found in the serological examinations of the patients' serum, and in one case at least were proved to be incorrect on ascertaining the agglutinnogenesis of the infective strain

- 4 Agglutinnogenesis experiments and absorption tests are probably required for a definite typing of most strains of *B. flexner* except old laboratory cultures
- 5 Inagglutinability and lack of agglutinnogenetic power appear to be closely related
- 6 Evidence of at least one strain with apparently no antigenic relationship to *B. flexner* V, W, X, Z and Y, has been found, and there are probably many others

TABLE II—(concluded)

Case No	Number of days under treatment	Cholesterol content per cent
13	5 days	0.12
14	5 days	0.109
15	4 days	0.109
16	4 days	0.096
17	9 days	0.109
18	9 days	0.104
19	14 days	0.096
20	14 days	0.109

#### Method of analysis employed

The method employed for the estimation of the cholesterol content of whole blood was that described by Myers and Wardell. One c.c. of oxalated whole blood was accurately measured by means of a pipette and allowed to flow drop by drop over four to five grams of plaster of Paris, contained in a porcelain dish. After fifteen to twenty minutes the blood was intimately incorporated with the plaster of Paris by the aid of a small glass rod. We noticed that if stirring was started immediately after the addition of the blood, it was difficult to free the end of the glass rod from adhering blood. This difficulty we found could be obviated by allowing the plaster to soak up the blood for the time stated before commencing to stir.

After thorough incorporation of the mixture it was transferred in the porcelain dish to the hot air oven (Temperature 100°C) for about two hours, the dish being removed from time to time and the coarser particles broken up. Finally the dish was removed from the hot air oven and the contents thoroughly powdered and then transferred into a filter paper extraction cell.

The extraction cell was placed in an all glass Soxhlet apparatus and extracted with chloroform for two hours. When the volume of the chloroform extract in the flask was about 10 to 15 c.c. it was removed from the water bath, cooled, and filtered through a small filter paper into a 25 c.c. stoppered cylinder. The flask was subsequently washed twice with small amounts of chloroform which were passed through the filter paper and added to the main filtrate. Finally the filter paper was washed with chloroform poured over it drop by drop until the volume of the filtrate reached 20 c.c. The combined filtrates were then well mixed and 5 c.c. pipetted into a clean dry test tube marked C. Into another clean dry test tube marked S 5 c.c. of a standard cholesterol solution (containing 0.4 mg. cholesterol) were pipetted. To each of the test tubes separately were then added 2 c.c. of acetic anhydride and 0.1 c.c. pure concentrated sulphuric acid and the contents well mixed. The tubes so treated were placed in a beaker containing water and kept in a dark place for ten minutes to allow the colour to develop. Colour matching

# NOTES ON THE CHOLESTEROL CONTENT OF INDIAN BLOOD IN HEALTH AND IN LEPROSY

BY

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THE investigation of the cholesterol content of the blood both in the normal and pathological state appears to be assuming greater importance every day, partially due to the fact that its exact rôle in the biochemical tangle of metabolism is as yet little understood and also to the stimulus received from the works of Hess and Weinstock and Rosenheim and Webster showing the close association of cholesterol with the vitamine fraction

The administration of hydnocarpus oil or its preparations in large doses such as given in the treatment of leprosy might reasonably owe its beneficial action to

(1) The presence of some activated body associated with the sterols of the oil, or

(2) Some alteration of the lipid metabolism of the body

It was possible then that any alteration caused by their administration might be reflected in the cholesterol content of the blood

It was from a consideration of these points of view that we decided to undertake an investigation of the cholesterol content in the blood of lepers, before and after treatment

The first difficulty that we encountered in the matter was the question of deciding on a normal standard for the cholesterol content of whole blood. European and American figures were certainly available but we were unable to accept these for the population that we had to deal with in this part of India, where the environment and mode of life differed so considerably from that of the types mentioned.

(2) Advanced skin cases Results shown in Table IV Average content 0.08 per cent with a maximum of 0.109 and a minimum figure of 0.07

TABLE IV  
*Advanced Skin Cases*

Case No	Initials	Mark	Cholesterol content per cent
1	P	B <sub>2</sub>	0.07
2	K K	B <sub>2</sub>	0.08
3	M	B <sub>2</sub>	0.07
4	N	B <sub>2</sub>	0.076
5	S K O	B <sub>2</sub>	0.07
6	K Ch D	B <sub>2</sub>	0.08
7	K B	B <sub>2</sub>	0.10
8	U Ch C	B <sub>2</sub>	0.07
9	K	B <sub>2</sub>	0.085
10	N	B <sub>2</sub>	0.085
11	C B	B <sub>2</sub>	0.086
12	E L	B <sub>2</sub>	0.08
13	N	B <sub>2</sub>	0.075
14	R G	B <sub>2</sub>	0.084
15	N D	B <sub>2</sub>	0.10
16	A L		0.085
17	L B		0.07
18	A E D		0.109
19	D	B <sub>2</sub>	0.07

(3) After treatment cases Results shown in Table V Average figure 0.095 per cent with a maximum and minimum figure of 0.109 and 0.08

TABLE V  
*After Treatment Cases*

Case No	Initials	Mark	Cholesterol content per cent
1	S K	A T case under treatment since 26th May, 1926	0.109
2	R M	B <sub>2</sub> Treated since 2nd November, 1926 Wassermann positive	0.096
3	L	Treated since August 1926	0.088
4	L	Long under treatment	0.109
5	B	B <sub>2</sub>	0.090
6	L	B <sub>2</sub>	0.10
7	K M G		0.10
8	A K M		0.09
9	N C S		0.092
10	J N P		0.08

TABLE I—(contd)

Case No	Initials	Cholesterol content per cent	RE MARKS
27	P K R	0 109	Hindu male
28	B	0 109	Ditto
29	D	0 101	Ditto
30	B	0 109	Ditto
31	L	0 10	Ditto
32	U	0 12	Ditto, age 18
33	S K	0 109	Md male, age 30
34	P	0 126	Hindu male, age 30
35	J D	0 086	Ditto, age 22
36	R	0 13	Ditto
37	A	0 10	Ditto, age 15
38	B	0 10	Ditto, age 30
39	M B	0 109	Md male
40	SK I	0 095	Ditto
41	N P	0 101	Hindu male
42	B M	0 096	Ditto
43	S M	0 109	Ditto
44	G R	0 109	Md male
45	S A	0 114	Ditto
46	M I	0 09	Ditto
47	K H	0 129	Ditto
48	C	0 104	Hindu male
49	D K	0 114	Ditto
50	S Ch K	0 136	Ditto
51	S K M	0 11	Ditto
52	E A	0 16	Md male
53	T S	0 126	Hindu male
54	G K C	0 138	Ditto
55	E U	0 109	Md male
56	S L	0 109	Hindu
57	J	0 107	Ditto



TABLE I—(concl'd)

Case No	Initials	Cholesterol content per cent	REMARKS
89	R D	0 126	Hindu
90	K K D	0 110	Ditto
91	J S	0 101	Ditto
92	G R	0 096	Ditto
93	S M	0 120	Md
94	K	0 110	
95	I	0 109	
96		0 096	
97		0 160	
98	M B	0 12	Md
99	B N	0 126	
100		0 111	Hindu

It may be of interest to note here that during this routine research on the normal we examined many cases undergoing anti-rabic treatment thanks to the kindness of Dr Fox, the Director of the Pasteur Institute, Calcutta. These results are shown in Table II.

TABLE II  
Cases under Anti-rabic Treatment

Case No	Number of days under treatment	Cholesterol content per cent
1	1 day	0 096
2	5 days	0 09
3	10 days	0 13
4	14 days	0 14
5	10 days	0 10
6	10 days	0 10
7	14 days	0 12
8	14 days	0 126
9	5 days	0 114
10	5 days	0 109
11	1 day	0 109
12	1 day	0 10



seldom much longer than its stem, sixth vein ending further from the base of the wing than the base of the second vein

The scaling of the head and scutellum, the length and form of the male palpi, and the ornamentation of the body and legs, are extremely diverse, and these characters do not form a sure guide as to whether a given species may belong to *Aedes* or not

As in the Anophelini the grouping of species into subgenera is based mainly on the structure of the hypopygium of the male, and a knowledge of the morphology of the genitalia is now essential to the student of the Culicidae

Of the groups now treated as subgenera of *Aedes* the following have been dealt with in previous parts of this Revision —*Stegomyia* (Barraud 1923 (1), 1923 (3), 1927), *Christophersomyia* (Barraud 1923 (1) ), and *Finlaya* (Barraud 1923 (2), 1923 (3), 1923 (4), 1924 (1), 1924 (2), 1924 (3), 1924 (4), 1927) The reader is referred to these papers for the characters distinguishing each group

The Indian species belonging to the subgenera *Aedunorphus*, *Ochlerotatus*, and *Banksimella*, are described in this paper, and those belonging to the subgenera *Skusea* and *Aedes* (sens stric) will be described in the next part

*Aedes* (*Stegomyia*) *variegatus* (Dol) has now to be added to the Indian list as specimens were obtained in the Andaman Islands in 1926 Some notes on the species are given below

### ***Aedes* (*Stegomyia*) *variegatus* (Dol)**

*Culex variegatus*, Doleschall, 1858, *Nat Tijd Ned Ind*, Vol XVII, p 77

*Culex scutellaris*, Walker, 1859, *Proc Linn Soc London*, Vol III, p 77

*Culex zonatipes*, Walker, 1861, *Proc Linn Soc London*, Vol V, p 229

*Stegomyia pseudoscutellaris*, Theobald, 1910, *The Entomologist*, Vol XLIII, p 156

This species resembles the common *Aedes* (*Stegomyia*) *albopictus* (Skuse), but differs in the following particulars amongst others —there is a patch of flat silvery white scales over the root of the wing which is not present in *A. albopictus*, there are two lines of similar scales across the pleurae instead of a number of irregular patches, the dorsal white abdominal markings are removed from the base of each segment and curve towards the middle of each tergite, sometimes forming complete bands, the scutellum bears flat snowy-white scales on all the lobes, but there are a few black scales at the apex of the mid lobe The specimens from the Andaman Islands appear to be intermediate in some respects between the type form and three of the varieties described by Edwards from various Islands in the Pacific, but are distinct from var *andrewsi* Edw, from Christmas Island, south of Java, which is the only other locality within the Oriental Region where this mosquito has been found previously The following characters are present in the specimens from the Andamans —white dorsal bands on the abdomen complete or only very slightly interrupted in the middle, third and following sternites with black apical bands, hind femur with a large white spot at the tip, basal white ring on the fourth hind tarsal segment occupying

was carried out in a Kober Klett colorimeter the standard being set at 15 mm  
Standard cholesterol solution

This was prepared by dissolving 0.160 gram of pure cholesterol (Kahlbaum) in 100 c.c. of pure redistilled chloroform. 5 c.c. of this solution diluted to 100 c.c. with pure chloroform was used as our working standard.

5 c.c. = 0.4 mg. cholesterol

From the figures obtained from our examination of normals we found that we should have to consider a somewhat lower standard than that accepted for American and European populations, as our average worked out at 0.116 per 100 c.c. of whole blood with a maximum of 0.181 and a minimum figure of 0.082.

Having established then a figure for the population under review we proceeded to examine the cholesterol content of the whole blood taken from cases of leprosy using exactly the same technique but dividing the cases into three categories. We owe our thanks to Dr. Muir of the Calcutta School of Tropical Medicine for supplying us with the necessary specimens of leprosy blood.

(1) Early cases. The results of this investigation are shown in Table III. Average content—0.099 per cent with a maximum and minimum range of 0.13 and 0.08.

TABLE III  
Early Cases

Case No	Initials	Mark	Cholesterol content per cent
1	H R	A <sub>1</sub>	0.096
2	G H	A <sub>1</sub>	0.096
3	F R	A <sub>1</sub>	0.10
4	D	A <sub>1</sub>	0.104
5	B	A <sub>1</sub>	0.08
6	B B	A <sub>1</sub>	0.101
7	B M	A <sub>1</sub>	0.09
8	B	A <sub>1</sub>	0.09
9	R	A <sub>1</sub>	0.104
10	D	A <sub>1</sub>	0.088
11	K M	A <sub>1</sub>	0.087
12	R D	A <sub>1</sub>	0.101
13	R S	A <sub>1</sub>	0.11
14	S	A <sub>1</sub>	0.12
15	R S	A <sub>1</sub>	0.096

The initials under the word 'mark' mean the following —

- A<sub>1</sub> Early nerve case
- B<sub>1</sub> Advanced skin case
- B<sub>2</sub> More advanced skin case

(e) *pallidostratus* group *A pallidostratus*, *A mediolineatus*, and *A nigrostratus* general colouration yellowish or golden, in the first two there is a pair of submedian pale lines on the mesonotum, and in the last there are black stripes in this position. The legs of *A pallidostratus* are marked with longitudinal pale stripes.

(f) The remaining three species, *A littoralis*, *A culicinus*, and *A nummatus*, are somewhat aberrant forms. In all three the legs are brown and unornamented. In *A littoralis* the general colouration is yellowish and there are violet-black scales on the dorsum of the abdomen. *A culicinus* resembles *Culex fatigans* in general appearance but may be distinguished by the shape of the terminal segments of the abdomen in the female, and by the form of the palpi in the male. *A nummatus* resembles *Aedes (Finlaya) nuneus* in the presence of a round patch of white scales on the front of the mesonotum, the palpi of the male are only one-half the length of the proboscis, the tip of the abdomen of the female is less pointed than in the other species of the subgenus, the eighth segment not completely retracted and the cerci comparatively short. It is interesting to note that, as far as is known, this species is the only one of the subgenus which breeds in tree-holes and it is possible that the differences in structure may be correlated with this habit.

The subgenus *Ochlerotatus* is represented by only a few species in India and these appear to be confined to the extreme north-west and represent intrusions from the Palearctic Region. Two species are described in this paper whose range of distribution extends into India proper, but there are other species occurring in Baluchistan which have not been fully investigated at present. In 1923 Colonel L. G. Browse, I.M.S., very kindly made a small collection for me in this region at elevations from 7,300 to 14,000 feet above sea-level. This collection includes three or four species of *Ochlerotatus*, one of which is *A (O) caspius*, described in this paper, and one of the others appears to be *A (O) cataphylla* Dyar. Unfortunately, the collection included no males and some of the specimens are not in good condition so that at present I have not been able to identify them with certainty, and have been waiting for an opportunity to obtain more material.

The adults of the subgenera *Aedimorphus* and *Ochlerotatus* are so similar in general appearance that I have included the species of both in one synoptic table. There are however, distinct differences in the structure of the male genitalia by which the representatives of the two subgenera may be distinguished. These differences may be briefly stated as follows—*Aedimorphus* clasper usually highly specialised and of different form in each species, anal surface of side-piece with a basal lobe bearing hairs or processes, dorsal border of side-piece without a lobe at the base, phallosome divided into lateral plates bearing small teeth (Plate LVI, fig 1, Plate LVII, figs 7 to 10, Plate LVIII, figs 17 to 27, Plate LIX, figs 33 and 34). *Ochlerotatus* clasper long and simple with a terminal appendage, a well-developed harpago arising from the harpaginal fold, dorsal border of the side-piece with a lobe at the base (Plate LVI, fig 2), phallosome smooth and scoop-shaped without lateral plates or teeth.

SUMMARY AND CONCLUSIONS

(1) That the cholesterol content of the whole blood taken from the population in and around Calcutta must be considered as slightly lower than the usually accepted European and American figure

(2) That the cholesterol content of the blood of lepers is reduced in the early stages of the disease

(3) That in advanced skin cases the content is much more reduced

(4) That in advanced treated cases the blood cholesterol content does not return to normal

(5) That anti-rabic treatment has no effect on the cholesterol content of whole blood

The expense of this work has been defrayed from a grant received from the Indian Research Fund Association

REFERENCE

MYERS and WARDELL (1918)

*Jour Biol Chem*, Vol XXXVI, p 147

- 13 Pale scales on anterior surface of mid femur evenly sprinkled, basal lobe of male genitalia with numerous hairs *verans* (North India, Assam, and Burma)
- Pale scales on anterior surface of mid femur arranged in definite spots forming two longitudinal lines, basal lobe of male genitalia with one long hair and four long leaf-like processes *fisheri* sp n (Bombay Deccan and Coorg)
- 14 Some of the scutellar scales broad and white *caecus*  
All the scutellar scales narrow and yellow *imprimens*
- 15 A large patch of white scales on the front of the mesonotum *nummatus*  
No patch of white scales on the mesonotum 16
- 16 Dorsum of abdomen marked with violet-black apical bands (male), or almost entirely covered with violet-black scales (female) *littoralis*  
Dorsum of abdomen dark brown with well-marked basal creamy bands *culicinus*  
Dorsum of abdomen with the base and sides of each segment ochreous with a triangular dark area in the middle (male), or with an ochreous median stripe bordered on each side with dark brown (female) *mediolineatus*

Short descriptions of the species, with references, and distribution, as far as it is known, are appended, but the reader should also refer to the remarks on the general characteristics of the subgenera, and grouping of species, given above

### ***Aedes (Aedimorphus) lowisi* (Theo)**

*Reedomyia lowisi*, Theobald, 1910, *Mon Cul*, Vol V, p 257

Female scutellum covered with flat silvery scales contrasting with the rich brown colour of the mesonotum, dorsum of abdomen brown with narrow basal white bands, silvery spots at the tips of the femora and tibiae, narrow basal and apical pale rings on the hind tarsi, 5th segment pale

I have not seen a male

There is one female in the Central Malaria Bureau collection, Kasauli, from the Andaman Islands (*Lowisi*) Theobald gives Galle, Ceylon (*Bainbrigge Fletcher*) as another locality but the specimen to which this referred was later found to be *A jamesi* by Edwards

The type male and female are in the British Museum

### ***Aedes (Aedimorphus) jamesi* (Edw)**

*Ochlerotatus jamesi*, Edwards, 1914, *Bull Ent Res*, Vol V, p 77

Male and female Mesonotal scales dark brown with pale scales forming indefinite spots in some specimens, scutellar scales flat and silvery, abdomen dark brown with narrow basal pale bands and lateral basal pale patches, silvery spots at the tips of the femora and tibiae, hind tarsi with narrow basal white rings on

# A REVISION OF THE CULICINE MOSQUITOES OF INDIA

## Part XXIII.

THE GENUS *Aedes* (SENS LAT) AND THE CLASSIFICATION OF  
THE SUBGENERA DESCRIPTIONS OF THE INDIAN  
SPECIES OF *Aedes* (*Aedimorphus*), *Aedes*  
(*Ochlerotatus*), AND *Aedes* (*Banksinella*),  
WITH NOTES ON *Aedes* (*Stegomyia*)  
*Variegatus*

BY

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SINCE the commencement of the present Revision I have had opportunities of making an intensive study of many thousands of mosquitoes from all parts of India, and have come to the conclusion that it would be advisable, in order to avoid further confusion in the classification of the Tribe Culicini, to adopt the views of Edwards and of Dyar, and to treat various groups as subgenera of *Aedes*, instead of maintaining them as separate genera as was done in my earlier papers. The classification referred to is now generally accepted and is justified on the grounds that there is considerable difficulty in making any clear-cut division between the various groups of *Aedes* owing to the presence of a number of intermediate forms. These intermediate and aberrant species certainly appear to afford clear evidence that the different groups are actually closely related.

The genus *Aedes*, as a whole, may be characterised as follows—pulvilli absent, spiracular bristles absent, post spiracular, sterno-pleural, and upper mesepimeral bristles present, lower mesepimeral bristles usually absent, but a few are present in some forms, prothoracic lobes well separated, proboscis moderately slender, straight, and of uniform thickness, wing scales not unusually broad nor asymmetrical, palpi of the female not more than one-quarter the length of the proboscis, eighth segment of abdomen of female retractile, in some groups completely hidden within the seventh, particularly in dried specimens, postnotum without setae, anterior fork-cell of wing not unusually short, but

Until recently the Central Malaria Bureau collection did not include any specimens, but one female in very fine condition has been presented by Mr Bainbrigge Fletcher, from Pusa, Bihar, 21 vii 1927 (*Shaffi* coll.), from which the above description has been made. I have seen one other female specimen in the Indian Museum, from Kierpur, Purneah district, Bihar, 8—14 ix 1915 (*C. Pāva*).

The types are in the British Museum, from 'India' (*James*).

### ***Aedes (Aedimorphus) vexans* (Mg)**

*Culex vexans*, Meigen, 1830, *Syst. Besch.*, Vol. VI, p. 241

I have devoted some time to the study of this species and find that although it has a wide distribution through northern India and Assam, and extends into Burma, it is apparently replaced in southern India and Ceylon by two other species. These two species, whilst resembling *A. vexans* very closely in general appearance, differ from that species, and from one another, in certain details of structure and markings. These differences are set out in the synoptic table and are repeated again under the descriptions.

One of the species which I consider distinct is, I feel sure, Theobald's *Culex stenoctrus*, which was sunk under *A. vexans* by Edwards (1917). Under the description of *A. stenoctrus* which follows I have given my reasons for reinstating it as a valid species. The other species appears to be new and I have described it under the name *Aedes (Aedimorphus) fisheri*. There is the possibility that it may be another of Theobald's species now sunk under *A. vexans* but as it is distinct in markings and structure from that species and from *A. stenoctrus* this appears to be unlikely.

Through the kindness of Mr F. W. Edwards I have been able to examine a British male of *A. vexans* and this agrees with the common Indian species ranging through northern and eastern India.

As regards Indian specimens the distinguishing characters of *A. vexans* are as follows —a brownish mosquito with narrow basal pale bands on the tarsi, pale basal bands on the dorsum of the abdomen, femora speckled with pale scales especially the anterior surface of the mid femur, where the pale scales are usually numerous and evenly sprinkled, palpi of the male longer than the proboscis by about the length of the terminal segment, a pale ring at about the middle of the long segment, white scales dorsally at the bases of the last two segments, hair tufts yellowish, palpi of the female about one-sixth the length of the proboscis, brown with pale scales at the tips, proboscis of the male with an indefinite pale area at about the middle, especially beneath, that of the female extensively pale beneath and at the side, brown above with scattered pale scales, scutellar scales narrow and yellowish, claws of the hind tarsi of both sexes simple (possibly toothed in occasional specimens), postnotum usually dark brown. There is some variation in the shape of the pale abdominal bands but they are nearly always narrower in the middle than at the sides especially in the male. I have seen some specimens in which the bands are completely divided into lateral patches, in others there are median apical pale patches tending to form a longitudinal stripe. Male genitalia

about two-thirds of the length, middle part of the ninth tergite of male genitalia slightly convex, clasper moderately swollen at the tip, basal lobe of side-piece flat-ended with hairs at the apex all about the same length, a few of the hairs are stouter and more blade-like than the majority, side-piece nearly four times the length of the width at the base in a dorsal or ventral view (Plate LVI, figs 4 and 5) These characters are variable in other forms of the species and have been used to separate the different varieties referred to above

A few specimens were bred by my laboratory assistant, Sobha Ram, whilst assisting Major G. Covell, I.M.S., in a malarial survey of the Andamans in July 1926

The subgenera *ALDIMORPHUS* and *OCHLEROTATUS*

*Aedimorphus*, Theobald, 1903, *Mon. Cul.*, Vol III, p. 290

*Ochlerotatus*, Arribalzaga, 1891, *Rev. Mus. La Plata*, Vol II, p. 143

The mosquitoes belonging to these subgenera may, in the females, be distinguished fairly easily from those of other genera by the shape of the terminal segments of the abdomen. The eighth segment is small and usually completely retracted within the seventh in repose, the cerci are well developed and project from the tip of the seventh segment (compare Plate LIX, figs 36 and 37)

In the males the palpi are usually as long as, or slightly longer than the proboscis, the terminal segments thickened, turned downwards and with hair tufts. The antennal hairs in the males are arranged chiefly on the dorsal and ventral aspects, and not in regular whorls as in the males of most other mosquitoes

There are no lower mesepimeral bristles, the number of pro-epimeral is usually 6 or 7, but in *A. mediolineatus* there are about 11, and in *A. nummatus* there are only two or three

The subgenus *Aedimorphus* has a wide distribution in India and includes 17 recognised species. *A. vexans* is a troublesome blood-sucker in some parts of the country. The species may be conveniently arranged in the following groups —

(a) *alboscuteclatus* group *A. alboscuteclatus*, *A. nivoscutella*, *A. lowi*, and *A. jamei*, scutellum covered with flat brilliant silvery scales contrasting remarkably with the brown or reddish brown colour of the mesonotum

(b) *vexans* group *A. vexans*, *A. stenoctus*, and *A. fisheri* sp. n., brownish mosquitoes with pale basal bands on the tarsi, femora speckled with pale scales, basal pale bands on the abdomen. These species are distinct from all the others in the structure of the male genitalia. In the form of the basal lobe, the plates of the anal segment, and the phallosome, this group closely resembles the subgenus *Stegomyia*

(c) *caecus* group *A. caecus*, and *A. impudens*, very similar in appearance to the last group but the femora are not speckled with pale scales. In the structure of the male genitalia these species appear to be more closely to the other groups

(d) *pipersalatus* group *A. pipersalatus* and *A. taeniorhynchoides*, wings and legs plentifully sprinkled with pale scales



The characters by which I distinguish *A. stenoetrus* from *A. verans*, based on the examination of five males and twenty females, are as follows —both tarsal claws on all the legs toothed in the male and female, proboscis of the male entirely dark brown, palpi of the male shorter than in the male of *A. verans* — being longer than the proboscis by only half the length of the terminal segment or less, the hair tufts also appear browner than in *A. verans*, clasper of the male genitalia with a longer and more pointed appendage than in either *A. verans* or *A. fisheri*, basal lobe larger and with more numerous hairs (compare Plate LVII, figs 7, 8 and 9), postnotum in both sexes usually pale brown or yellowish

Apart from the toothed claws, which are present in the twenty females examined, this sex is difficult to separate from the females of *A. verans*. The pale rings on the hind tarsi are well-marked and wider in some specimens than is usual in *A. verans*. The basal white abdominal bands are very slightly constricted in the middle in both sexes

The Central Malaria Bureau collection contains specimens from the Nilgiri Hills, 1915 (*Khazan Chand*), Coonoor, Nilgiri Hills (*Patton*), and Kodaikanal (*Patton*). There are no specimens of *A. verans* from these localities in the collection

I have not at present had an opportunity of examining any specimens from Ceylon and have relied on Theobald's descriptions

I am in doubt as to the identity of *Culcada eruthrosops* Theo the type female of which came from Trincomalee, Ceylon (*Green*). This place is on the north-east coast of the Island and has a drier and hotter climate than Maskeliya and Hakgala referred to above

The type females of *C. stenoetrus* and *C. pseudostenotruss* are in the British Museum

### ***Aedes (Aedimorphus) fisheri* sp. n.**

This species closely resembles *A. verans* and *A. stenoetrus*, but may be distinguished as follows —pale scales on the anterior surface of the mid femur of the female arranged in definite small spots, forming more or less two longitudinal rows, in the male these spots are usually less definite, palpi of male longer than the proboscis by nearly the length of the last segment, hair tufts pale brown or yellowish, basal lobe of male genitalia with one long hair and four long leaf-like processes (Plate LVII, figs 9 and 10), proboscis of the male with a pale area in the middle, the border of this area nearest to the base of the proboscis sharply defined, apical one-third of the proboscis of the female noticeably blackish-brown when viewed from above, the remainder dark brown speckled with pale scales, claws of the hind tarsi simple in both sexes, postnotum usually dark brown, basal white abdominal bands straight or very slightly narrower in the middle

I have examined 14 males and 16 females from Tavargatti, Bombay, Deccan, bred from larvae obtained from a jungle pool, August 1921 (*Barraud*), and two males from Somwarpet, Coorg, bred from larvae found in a small grassy pool, June 1927 (*Sub-Assistant Surgeon J. D. Baily, I.M.D.*). The 30 specimens bred from one batch of larvae from the same pool, mentioned

Synoptic Table for the identification of the adults of the Indian species of  
*ARDES* (*ARDEMORPHUS*) and *ARDES* (*OCHIROTATUS*)

1	Scutellum completely covered with flat silvery scales contrasting with brown or reddish brown colour of mesonotum	2
	Scutellar scales not silvery, usually narrow and yellow (some rather broad white scales in <i>piperisulatus</i> and <i>caccus</i> )	4
2	Hind tarsi with basal white rings	<i>jamesi</i>
	Hind tarsi with basal and apical rings	<i>lovisi</i>
	Hind tarsi entirely dark	3
3	Abdominal tergites with pure white basal lateral spots, and sometimes complete white bands	<i>alboscutecllatus</i>
	Abdomen with a continuous yellow lateral stripe on each side	<i>macroscutella</i>
4	Wings speckled with pale scales	5
	Wings not speckled	7
5	Hind tarsi marked with apical and basal white rings, mesonotum bright fawn colour with a pair of paler stripes	<i>O caspius</i>
	Hind tarsi marked with narrow basal pale rings only, mesonotum without pale stripes	6
6	Pale scales of mesonotum forming roundish spots, some broad white scales on mid lobe of scutellum	<i>piperisulatus</i>
	Pale scales of mesonotum numerous and evenly distributed, not forming spots, narrow pale scales on mid lobe of scutellum	<i>lacmorhynchoides</i>
7	Femora and tibiae marked with longitudinal pale stripes	<i>pallidostratus</i>
	Femora and tibiae not striped	8
8	Legs almost entirely bright golden yellow, mesonotum marked with a pair of black stripes, a black spot over each wing root	<i>ingrostratus</i>
	Legs not bright yellow, thorax without black stripes	9
9	Hind tarsi marked with pale rings	10
	Hind tarsi without pale rings	15
10	Basal and apical rings on hind tarsi, 5th segment entirely white	<i>O pulchritarsis</i>
	Basal pale rings on hind tarsi, 5th segment not entirely white	11
11	Anterior surface of mid femur speckled with pale scales	12
	Anterior surface of mid femur dark brown without a speckling of pale scales	14
12	Both claws of hind tarsi toothed in both sexes, proboscis of male entirely dark brown, postnotum usually light brown or yellowish, clasper of male genitalia with a rather long appendage (Plate LVII, fig 8)	
	Claws of hind tarsi simple in both sexes, proboscis of male with a pale area in the middle, postnotum usually dark brown, clasper of male genitalia with a shorter appendage (Plate LVII, figs 7 and 9)	<i>stenoctus</i> (Mountains of south India and the higher parts of Ceylon)
		13 -

female of *C. suknaensis* and two co-type females from Sukna, North Bengal, July 1908 (*Annandale*) All these appear to be the same species but the form of the scutellar scales certainly varies a good deal and I am inclined to think that Theobald's *Culex caecus* and *Culcada suknaensis* are possibly conspecific, particularly as there appears to be very little difference, if any, in the structure of the male genitalia The male of Walker's *Culex imprimens* is unknown and this species was described from a female from Amboina, an island east of Celebes, and it is possible that it may eventually be found to be distinct from the Indian species

The 50 specimens referred to above were obtained from the following localities —Bengal, Rangamati, Chittagong Hill Tracts, September 1922 (*Barraud*) Assam, Dimapur and Dibrugarh, July and August 1922 (*Barraud*), Golaghat, January 1925 (*Barraud*)

The type female of *C. imprimens* is in the British Museum, and that of *C. suknaensis* in the Indian Museum

### ***Aedes (Aedimorphus) pipersalatus* (Giles)**

*Stegomyia pipersalata*, Giles in Theobald, 1901, *Mon Cul*, Vol II, p 31b, (female only)

*Pseudograbhamia maculata*, Theobald, 1905, *Jour Bomb Nat His Soc*, Vol XVI, p 243

This and the following species may be distinguished from the other members of the subgenus by the speckling of pale scales on the wings, proboscis, and legs In *A. pipersalatus* the mesonotum is dark brown with pale scales forming small round spots, and scutellar scales are white and rather broad especially those on the mid lobe, these characters serve to distinguish this species from *A. taeniorhynchoides* There are narrow basal pale rings on all the segments of the hind tarsi in both species

*A. pipersalatus* appears to have an eastern and southern distribution, whereas, as far as is known, *A. taeniorhynchoides* is confined to the Punjab and Delhi Province I have examined specimens of the former from the following localities —Central Provinces, Kamptee, Nagpur district, Bombay Presidency, Belgaum and Tavargatti, August 1921 (*Barraud*) Bihar, Ranihat, Cuttack (*S Sundar Rao*), Pusa, 31 vii 1927 (*Shaffi* coll) In some notes kindly supplied to me by Mr F W Edwards the following additional records are given —United Provinces, Anwarganj, Cawnpore district (*J W C*) Bengal, Madhupur (*C Parva*)

Giles' description of *Stegomyia pipersalata* was a composite one, the male and female belonging to different species The female is taken to be the type and is labelled Gonda (United Provinces) *Pseudograbhamia maculata* was described from specimens from Ceylon (*Green*), and Theobald gives Madras Town as an additional locality The type specimens are in the British Museum

the 2nd to the 5th segments. Palpi of the male longer than the proboscis by a little more than the length of the apical segment. The clasper of the male genitalia is similar to that of *A. alboscuteUatus* but less expanded at the extremity (Plate LVIII, fig 25).

Specimens are in the Central Malaria Bureau collection, Kasauli, from Madras (Patton), Tavargatti, Bombay Deccan, larvae collected from pools in jungle, August 1921 (Barraud). I have seen one male and three females collected at Pusa, Bihar. Specimens are in the British Museum from Colombo, Ceylon (James) (including type female), and Galle, Ceylon (Bambridge Fletcher).

### ***Aedes (Aedimorphus) alboscuteUatus* (Theo)**

*Lepidotomyia alboscuteUata*, Theobald, 1905, *Ann Mus Nat Hung*, Vol III, p 80

*Reedomyia alboscuteUa*, Theobald, 1907, *Mon Cul*, Vol IV, p 261

*Reedomyia pampangensis*, Ludlow, 1905, *Can Ent*, Vol XXXVII, p 94

*Culex argentimotus*, Banks, 1909, *Phil Jour Sci*, Vol IV, p 547

*Aedes omurensis*, Yamada, 1921, *Annot Zool Jap*, Vol X, p 73

Male and female. Thorax rich dark brown, scutellar scales flat and brilliantly silvery, lateral basal white patches on the abdominal tergites not usually visible dorsally, narrow basal pale bands in some specimens, silvery spots at the apices of the femora and tibiae, tarsi entirely dark. Palpi of the male longer than the proboscis by nearly the length of the last two segments. The form of the clasper of the male genitalia is shown in Plate LVIII, fig 24.

I have examined specimens from Tavargatti, Bombay Deccan, bred from larvae found in jungle pools, August 1921 (Barraud), and from Golaghat, Upper Assam Valley, December 1924, and January 1925, caught in jungle (Barraud). The type female of *L. alboscuteUata* is in the National Museum of Hungary, Buda Pest, from Papua (Buó), the type male and female of *C. argentimotus* are in the Entomological collection, Bureau of Science, Manila, from Rizal, Philippine Islands (Banks), that of *R. pampangensis* in the Army Medical Museum, Washington, from Anzeles, Pampanga, Philippine Islands (Whitmore). *A. omurensis* was described from Omura, Kiushu, Japan, 'four females and two males taken as the syntype (No 35).'

### ***Aedes (Aedimorphus) niveoscutella* (Theo)**

*Reedomyia niveoscutella*, Theobald, 1905, *Jour Econ Biol*, Vol I, p 22

Female. Mesonotum uniformly dark brown except for a narrow border of pale scales at the sides, scutellar scales brilliantly silvery, proboscis yellowish except at the extreme tip, all the legs appear pale golden yellow when seen from behind, mid femora dark brown when viewed in the opposite direction, anterior surface of hind femur pale yellow for the whole length, small silvery spots at the tips of the femora, tarsi entirely dark, dorsum of abdomen dark brown with a yellow strip down each side for the whole length, a few yellow scales on the dorsum on segments 5, 6, and 7.

dark scaled, legs yellowish or brownish without pale stripes or rings, proboscis dull yellow except at the extreme tip, palpi of the male yellowish, the apex of the long segment and the last two segments brownish above, hair tufts yellow, or brown when viewed from the side against a white background, the whole palpi is longer than the proboscis by a little more than the length of the last segment, palpi of the female about one-quarter the length of the proboscis. In the male the dorsum of the abdomen is marked with a triangular dark area on each tergite with the apex towards the base of each segment, yellow scales at the sides, in the female there is a median pale ochre-yellow stripe down the dorsum bordered on each side by dark brown, pale yellow again at the sides. The shape of the clasper of the male genitalia is shown in Plate LVIII, fig 20.

The types of *C. mediolineatus* and *C. trilineatus* are in the British Museum, both from Thayetmyo, Burma (Watson).

Edwards has given *Culex trimaculatus* (Theobald, 1905, *Ann Mus Nat Hung*, Vol III, p 86) as a synonym of this species, but the description, based on a female from Bombay, 1902 (Bnó), does not appear to agree in some points, and I have not seen any specimens from the western side of India. There are a few specimens of *A. mediolineatus* in the Central Malaria Bureau collection, from Thayetmyo, Burma, received 21 xi 1925, from the Superintendent of the Jail.

### ***Aedes (Aedimorphus) nigrostriatus* Barr**

*Aedimorphus nigrostriatus*, Barraud, 1927, *Ind Jour Med Res*, Vol XIV, p 594

This species was fully described under the reference given above.

The distinguishing characteristics are as follows — a golden yellow mosquito marked with a pair of conspicuous black stripes on the mesonotum and a round black spot in front of each wing base, proboscis, abdomen and legs almost entirely golden yellow. On the wings there are black scales on about the basal two-thirds of vein 1, on veins 2 and 4 from the cross-veins continued along the lower branch of 2 (2 2) and upper branch of 4 (4 1), along the stem of 5 and 5 2, other veins and costa yellow scaled. The clasper of the male genitalia is remarkably specialised (Plate LVIII, fig 17).

The type male and female are now in the British Museum, from Golaghat, Assam, 17 xi 1925 (Barraud), other specimens from the same place are in the Central Malaria Bureau collection, and one female from Rangoon (Capt R W Hamilton Miller).

### ***Aedes (Aedimorphus) littoralis* Barr**

*Aedimorphus littoralis*, Barraud, 1927, *Ind Jour Med Res*, Vol XIV, p 551

A full description of this species was given under the reference given above. Distinguishing characteristics — a yellowish mosquito without conspicuous ornamentation, legs brown and unbanded, dorsum of abdomen of male with violet-black apical bands, which are broad on segments 2, 3 and 4, abdomen of the

tip of clasper rather blunt, appendage shorter, and arising from a shorter prominence, than in *A. stenoetrus*, basal lobe smaller and with fewer hairs (compare Plate LVII, figs 7 and 8)

With regard to the synonymy of Oriental forms, after removing *Culex stenoetrus* and *Culex pseudostenoetrus*, the following names appear to remain sunk under *A. verans* — *Culicada nipponi*, Theobald, 1907, *Mon Cul*, Vol IV, p 337, described from one female from Kaniyama, Japan (Cornford); *Culicada minuta*, Theobald, 1907, *Mon Cul*, Vol IV, p 338, described from a female from India (Christophers), probably from Mian Mir, Lahore, *Culicada cruthersops*, Theobald, 1910, *Mon Cul*, Vol V, p 229, described from a single female (hind tarsi missing) from Trincomalee, Ceylon (Green) (note remarks under *A. stenoetrus*) The type females of the above-mentioned three species are in the British Museum *Culex vagans*, Theobald (nec Wiedmann) 1901, *Mon Cul*, Vol I, p 411, redescribed from one female and recorded from Hongkong and Shanghai, *Culex linsutum*, Ludlow, 1911, *Psychic*, Vol XVIII, p 126, ? *Culex auratus*, Leicester, 1908, *Cul Malaya*, p 153, described from the Malay Peninsula, type apparently lost

I have examined specimens of *A. verans* from the following places in India — N W Frontier, Kohat (Sinton) and Abbottabad, Central Province, Pachmar (V H Dowson), United Provinces, Bhowali, Naini Tal (S Sundar Rao), Assam, Golaghat and Khumtai (Christophers), Golaghat, October 1924, December 1924, January 1925 (Barraud), Shillong and Nongpoh, June and July 1922 (Barraud), Burma, Rangoon (Christophers)

### ***Aedes (Aedimorphus) stenoetrus* (Theo)**

*Culex stenoetrus*, Theobald, 1907, *Mon Cul*, Vol IV, p 395

*Culex pseudostenoetrus*, Theobald, 1910, 1 c, Vol V, p 343

This resembles *A. verans* very closely and has been considered as synonymous with that species. I have reinstated it as distinct on the characters given in the synoptic table. These include the presence of toothed claws on the hind tarsi in both sexes. This is a very unusual character in this subgenus and is not found in any of the other Indian species as far as I have observed. I am aware that the form of the claws is subject to variation and is not usually of great importance, but in this species it appears to be constant and is correlated with differences in the structure of the male, mentioned below, and with a definite distribution, viz, the mountains of south India and the higher parts of Ceylon. Theobald described *C. stenoetrus* from a female from Maskeliya, Ceylon (Green), and *C. pseudostenoetrus* from two females from Hakgala, Ceylon (Green), and there is little doubt that the two species are the same. Mr R. Senior-White has kindly informed me that Maskeliya is about 4,000 feet above sea, and Hakgala about 6,500 feet, and that the rainfall in these localities is about 200 inches per annum, as they are under the influence of both the north-east and south-west monsoons. The climate of the hills of south India is very similar. In his description of *C. pseudostenoetrus* Theobald distinctly states that all the tarsal claws are uniserrate (toothed)

**Aedes (Ochlerotatus) caspius** (Pallas)

*Culex caspius*, Pallas, 1771, *Reise versch Prov Russ Reichs*, Vol I, p 475

This species may be fairly easily distinguished by the characters given in the synoptic table —wings speckled with light and dark scales, tarsi marked with pale apical and basal rings, mesonotum clothed with bright fawn-coloured scales and marked with a pair of ill-defined white stripes running from the front of the thorax to the scutellum. It is a Palearctic species and up to the present has been found only in the extreme north-west of India. Drawings illustrating the structure of the male genitalia are shown in Plate LVI, figs 2 and 3.

I have seen specimens from Nowshera, vi 1911 (*Major Smith*), Bragica, Baltistan, 7,300 feet, viii 1923 (*Colonel L G Browne*), Bannu and Peshawar.

**Aedes (Ochlerotatus) pulchritarsis** (Rond)

*Culex pulchritarsis*, Rondani, 1872, *Bull Soc Ent Ital*, Vol IV, p 31

*Finlaya versicolor*, Barraud, 1924, *Ind Jour Med Res*, Vol XII, p 73

A description of the female of this species (under the name *F versicolor*) will be found in this Journal under the above-mentioned reference. It is said to be exclusively a tree-hole breeding species and it bears a strong resemblance to some species of *Finlaya* having similar habits. Up to the present I have not seen a male. Like *A caspius* it has only been found in the extreme north-west of India. It is a well-marked species and the distinguishing characters are as follows —general colouration black, brown, and white, fifth tarsal segment of all the legs entirely white, white basal and apical rings over the joints between the tarsal segments on all the legs except that between the third and fourth segments on the fore and mid pairs, a wide central area of bright golden brown scales on the front of the mesonotum, white basal bands to the abdominal tergites, wings dark scaled.

Var *asiaticus*, Edwards, 1926, *Riv Malariaologia*, Vol V, N S I, fasc 4, p 430

In this form there is a more or less distinct central white stripe on the mesonotum dividing the brown area. No locality was given in the description of this form, but presumably it referred to specimens sent to Mr Edwards by me from Drosh, Chitral (*Walker*).

The type of *F versicolor* is now in the British Museum from Yusimarg, Kashmir, 7,500 feet, 6—15 viii 1923 (*Bambingge Fletcher*).

## Aedes subgenus BANKSINELLA

*Banksinella*, Theobald, 1907, *Mon Cul*, Vol IV, p 468

**Aedes (Banksinella) lineatopennis** (Ludl)

*Taeniorhynchus lineatopennis*, Ludlow, 1905, *Can Ent*, XXXVII, p 133

*Culex lutcolateralis*, Theobald, 1901, *Mon Cul*, Vol II, p 71 (in part)

This is the only species of the subgenus at present known from India. It may be distinguished from other mosquitoes by the following characteristics —

above, are marked as co-types and are at present in the Central Malaria Bureau collection.

The larva (Plate LIX, figs 28 to 32) differs from the description of that of *A. veians* in having the mid post antennal hair, on the dorsal surface of the head, single and rather stout, the inner post antennal hair is four-branched. The chitinated tube of the siphon is about  $2\frac{1}{2}$  times longer than the greatest width, pecten 13 to 19 teeth, the two or three distal teeth larger, more widely spaced, and without distinct lateral denticles, comb of eighth abdominal segment composed of 10 rather large teeth. The above description has been made from the skins cast by three isolated larvae at the time of pupation and from which the resultant adults have been preserved.

I have much pleasure in dedicating this species to the late Dr R W Fisher, formerly Director of the Vaccine Institute, Belgium, who showed me much kindness and hospitality during my collecting tour in the Bombay Deccan in 1921.

### *Aedes (Aedimorphus) caecus* (Theobald)

*Culex caecus*, Theobald, 1901, *Mon Cul.* Vol I, p 413

*Peconomyia caeca*, Theobald, 1907, 1 c, Vol IV, p 268

This species resembles the *A. veians* group in general appearance but may be distinguished by the absence of pale scales on the femora, the anterior surface of the mid femur particularly is entirely dark brown. There are some rather broad white scales on the scutellum, sometimes on all the lobes, in other specimens the scales on the mid lobe are mostly narrow, this variation occurs in both sexes. The presence of these broad scales is about the only character by which this species may be distinguished from *A. imprimens* (Walk.) described below.

The pale rings on the tarsi are quite narrow, and the pale basal bands on the dorsum of the abdomen narrow and straight, proboscis dark brown with usually an indefinite pale area in the middle at the sides and beneath, palpi of the male longer than the proboscis by about the length of the last segment, a pale area at the base and a distinct pale ring at about the middle of the long segment, white scales dorsally at the bases of the last two segments, hair tufts brown, antennal hairs of the male brownish to yellowish. I can find very little difference in the structure of the male genitalia between this species and *A. imprimens*.

There are a small number of specimens in the Central Malaria Bureau collection from the Malabar Coast, 1915 (*Khazan Chand*).

*Culex caecus* was described from one female from Selangor (*Butler*), it was later placed in the genus *Peconomyia* by Theobald. The type is in the British Museum.

### *Aedes (Aedimorphus) imprimens* (Walker)

*Culex imprimens*, Walker, 1861, *Proc Linn Soc London*, Vol V, p 144

*Culicada suknaensis*, Theobald, 1910, *Rec Ind Mus*, Vol IV, p 21

This resembles *A. caecus* described above very closely. I have examined about 50 specimens from Bengal and Assam, together with Theobald's type.



# EXPLANATION OF PLATE LVI

Drawings illustrating the structure of the male genitalia in *Aedes* (*Aedimorphus*), *Aedes* (*Ochlerotatus*), *Aedes* (*Stegomyia*), and *Aedes* (*Banksinella*) All the drawings show the dorsal view (ventral after rotation of the hypopygium)

Fig 6 drawn to the scale shown under Fig 1

- Fig 1 *Aedes* (*Aedimorphus*) *imprimens* Side-piece (hairs omitted), clasper, basal lobe or plaque on the anal surface of side-piece, and indefinite lobe on ventral border
- „ 2 *Aedes* (*Ochlerotatus*) *caspus* Side-piece (hairs omitted), clasper, lobe at the base of dorsal border of side-piece, and harpago
- „ 3 *Aedes* (*Ochlerotatus*) *caspus* Harpago on a larger scale
- „ 4 *Aedes* (*Stegomyia*) *variegatus* (Andamans) Side-piece (hairs omitted), clasper, basal lobe or plaque on anal surface of side-piece, and small lobe on ventral border
- „ 5 *Aedes* (*Stegomyia*) *variegatus* Middle part of ninth tergite
- „ 6 *Aedes* (*Banksinella*) *lineatopennis* Side-piece (hairs of outer side omitted) clasper, hairs and spines on anal surface of side-piece, and lobe at base of dorsal border with spines

**Aedes (Aedimorphus) taeniorhynchoides** (Chris)

*Lesliomyia taeniorhynchoides*, Christophers, 1911, *Paludism*, No 2, p 68  
*Pecomyia maculata*, Theobald, 1905, *Ann Econ Biol*, Vol I, p 23

This species resembles *A. pipersalatus*, described above, but may be distinguished by the lighter colour of the mesonotum owing to the presence of more numerous pale scales which do not form spots, and by the narrower scales on the mid lobe of the scutellum, in other respects the ornamentation of the two species is very similar. There are small differences in the shape of the clasper of the male genitalia (Plate LVIII, figs 22 and 23)

*A. taeniorhynchoides* appears to be confined to the Punjab and Delhi Province, and I have examined specimens from the following places—Amritsar, September 1910, three specimens including the types (*Christophers*), Chandigarh, near Kalka, June 1914 (*Colonel C A Gill*), Ambala, August 1923 (*Capt K R K Iyenger*), New Delhi, vii 1927 (*R Senior-White*), Karnal, vii 1927 (*Barrand*)

The types of *L. taeniorhynchoides* are in the Central Malaria Bureau, and that of *P. maculata* in the British Museum, probably from Mian Mir, Lahore (*Christophers*)

**Aedes (Aedimorphus) pallidostriatus** (Theo)

*Culex pallidostriatus*, Theobald, 1907, *Mon Cul*, Vol IV, p 410

*Culex parascelos*, Theobald, 1910, *Rec Ind Mus*, Vol IV, p 18

Male and female femora and tibiae and first tarsal segments marked with conspicuous longitudinal pale stripes, mesonotum dull yellow with a pair of submedian paler stripes, front edge of costa of wing pale scaled for the whole length, dorsum of abdomen entirely covered with bright ochre-yellow scales. This is rather a large mosquito and owing to its yellowish colour and striped legs is distinct from any other member of the subgenus. The shape of the male clasper is shown in Plate LVIII, fig 18

I have seen specimens from the following places—Delhi, 15 viii 1927 (*R Senior-White*), Trombay (Bombay Harbour) July 1921 (*Barrand*), Cuttack, Bihar (*S Sundar Rao*), Madras, March 1914 (*Patton*). The following additional records are in some notes supplied to me by Mr F W Edwards—Bengal, Dum Dum (*M O T Iyenger*), Tinpahar, near Rajmahal (*Annandale*), Manhupar (*C A Parva*), United Provinces, Anwarganj, Cawnpore district (*J W C*)

The types of *C. pallidostriatus* are in the British Museum, from Peradeniya, Ceylon (*Green*) and India (*Christophers*), and that of *C. parascelos* in the Indian Museum, from Madras Town (*Mus coll*) 30 x 1908

**Aedes (Aedimorphus) mediolineatus** (Theo)

*Culex mediolineatus*, Theobald, 1901, *Mon Cul*, Vol II, p 113

*Culex trilineatus*, Theobald, 1901, 1 c, p 105

Male and female mesonotal scales yellowish, a pair of submedian paler stripes from the front of the thorax continued back to the scutellum, wings

## EXPLANATION OF PLATE LVII

Drawings illustrating the structure of the male genitalia in *Aedes* (*Aedimorphus*) and *Aedes* (*Ochlerotatus*)

Figs 7, 8, 9, and 11, drawn to the scale shown between Figs 7 and 8,  
Figs 12 to 16 drawn to the scale shown under Fig 15

Fig 7 *Aedes* (*Aedimorphus*) *veians* Basal lobe and clasper (slide preparations)

„ 8 *Aedes* (*Aedimorphus*) *stenoctrus* Basal lobe and clasper (slide preparation)

„ 9 *Aedes* (*Aedimorphus*) *fisheri* sp n Basal lobe and clasper (slide preparation)

„ 10 *Aedes* (*Aedimorphus*) *fisheri* Dorsal view (ventral after rotation of the hypopygium) of side-piece, hairs omitted, clasper, and basal lobe or plaque

„ 11 *Aedes* (*Aedimorphus*) *veians* Lateral view of clasper, showing abnormal double appendage seen in one specimen from Assam

Figs 12 and 13 *Aedes* (*Aedimorphus*) *caci* Plates of the anal segment (two sides of the same segment twisted in different positions), slide preparation

Fig 14 *Aedes* (*Ochlerotatus*) *caspus* Plates of one side of the anal segment, flat preparation

„ 15 *Aedes* (*Aedimorphus*) *veians* Plates of one side of the anal segment, flat preparation

16 *Aedes* (*Aedimorphus*) *nummatus* Plates of one side of the anal segment, flat preparation

female almost completely covered with violet-black scales dorsally. The clasper of the male (Plate LVIII, fig 27) is quite different in shape from that of any other Indian species of the subgenus.

The type male and allotype female are now in the British Museum, from Trombay, Bombay Harbour, July 1921 (Barraud).

### ***Aedes (Aedimorphus) culicinus* (Edw.)**

*Aedes (Ecculer) culicinus*, Edwards 1922, *Ind Jour Med Res*, Vol X, p 271

This species was described in this Journal under the reference given above. It is a brown mosquito with pale basal bands on the abdomen and unbanded legs, resembling *Culex fatigans*. The female may be distinguished from that species by the shape of the terminal segments of the abdomen, the eighth segment is usually completely hidden within the seventh, the cerci are long and project from the tip of the last named segment. The male may be distinguished from that sex of *C. fatigans* by the form of the palpi which are only a little longer than the proboscis, the terminal segments turned downwards and bearing hair tufts. The male genitalia are also, of course, quite distinct (clasper Plate LVIII, fig 19).

The type male is in the Central Malaria Bureau, and allotype female in the British Museum, both from Delhi, 1914 (Christophers). Another female specimen is in the British Museum from Amritsar, 1911 (Christophers), and there are a number of females in the C M B collection from Lahore, August and September 1923 (Sinton).

### ***Aedes (Aedimorphus) nummatus* Edw.**

*Aedes (Aedimorphus) nummatus*, Edwards, 1923, *Bull Ent Res*, Vol XIV, p 4

This is an aberrant species in markings and structure. In general appearance it resembles *Aedes (Fmlysa) niveus* (Ludl.) described and figured in Part VI of this Revision, especially in the presence of a large patch of white scales on the front of the mesonotum. The general colouration otherwise is dark brown, but the underside of the abdomen is pale. The hind femur is dull white on the outer or anterior side from the base to the tip, whereas in *A. niveus* the apical one-third is black. In the male the palpi are thin, without hair tufts and only about half the length of the proboscis. The tip of the abdomen in the female is rather blunt, the eighth segment not completely contracted and the cerci short. The clasper of the male (Plate LVIII, fig 21) is divided into two arms one of which carries a short terminal appendage.

The type male is in the British Museum, from Meenglas, Jalpaiguri (M. O. T. Iyengar). There are specimens in the Central Malaria Bureau from Bengal, Sukna, September 1922, bred from larvae found in tree-holes (Barraud), Assam, Nongpoh, July 1922, bred from larvae found in similar situations (Barraud), and Haflong, August 1922, bred from larvae taken from a broken bottle in jungle (Barraud).

# EXPLANATION OF PLATE LVIII

Drawings illustrating the shape of the clasper of the male genitalia of *Aedes* (*Aedimorphus*)

All the drawings are to the same scale

Fig 17	<i>Aedes</i>	( <i>Aedimorphus</i> )	<i>nigrostriatus</i>
„ 18	„	„	<i>pallidostriatus</i>
„ 19	„	„	<i>culcinus</i>
„ 20	„	„	<i>mediolineatus</i>
„ 21	„	„	<i>nummatus</i>
„ 22	„	„	<i>tacmorhynchoides</i>
„ 23	„	„	<i>pipersalatus</i>
„ 24	„	„	<i>alboscuteclatus</i>
„ 25	„	„	<i>jamesi</i>
„ 26	„	„	<i>imprimens</i>
„ 27	„	„	<i>littoralis</i>

general colouration dark brown, mesonotum marked with conspicuous lateral yellow stripes, yellow scales on some of the wing veins, legs dark brown and unbanded. *Mimomyia chamberlaini* Ludl (*Radioculex clavipalpus* Theo) has a rather similarly marked thorax but may be distinguished by the pale rings on the tarsi. In *Banksinella* the palpi of the male are longer than the proboscis, turned upwards at the extremity and apparently composed of only two segments. The male genitalia are distinctive, the form of the side-piece and of the clasper are shown in Plate LVI, fig. 6.

The single Indian species has a wide distribution and I have examined specimens from the following places—Lahore, Amritsar and Delhi (*Christophers*), Ambala and Karnal (*Barraud*), Pusa (*Capt A C Craighead*), Poona (*Assistant Health Officer*), Belgaum (*Barraud*), Malabar Coast (*Khazan Chand*), Golaghat, Assam (*Barraud*), Port Blair, Andaman Islands (*Christophers*).

*T. lineatopennis* was described from two specimens from Luzon, Philippine Islands (*Whitmore*). Theobald confused two species, one from Africa and one from the Malay Peninsula, in his description of *C. lutcolateralis*, and this name is reserved for the African species.

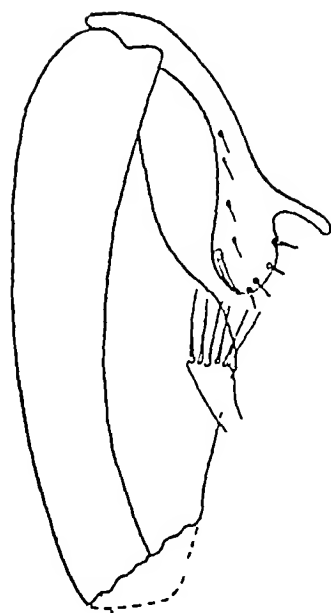
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|---------------------------|---|
| EDWARDS, F. W. (1917)     | <i>Bull. Ent. Res.</i> , Vol. VII, p. 218       |
| BARRAUD, P. J. (1923) (1) | <i>Ind. Jour. Med. Res.</i> Vol. X, pp. 772—788 |
| " " " (2)                 | <i>Ibid.</i> , Vol. XI, pp. 214—219             |
| " " " (3)                 | <i>Ibid.</i> , Vol. XI, pp. 224—228             |
| " " " (4)                 | <i>Ibid.</i> , Vol. XI, pp. 475—493             |
| " " (1924) (1)            | <i>Ibid.</i> , Vol. XI, pp. 845—865             |
| " " " (2)                 | <i>Ibid.</i> , Vol. XI, pp. 967—968             |
| " " " (3)                 | <i>Ibid.</i> , Vol. XI, pp. 999—1006            |
| " " " (4)                 | <i>Ibid.</i> , Vol. XII, pp. 73—74              |
| " " (1927)                | <i>Ibid.</i> , Vol. XIV, pp. 552—554            |

## EXPLANATION OF PLATE LIX

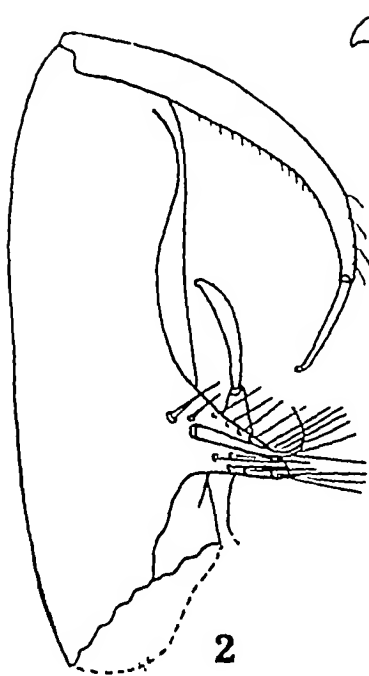
- Figs 28 and 29 drawn to the scale shown under Fig 28
- „ 30 and 31 drawn to the scale shown under Fig 32 marked (p)
- „ 32 to 35, 39 and 40 drawn to the scale shown under Fig 32 marked (c)
- „ 36 to 38 drawn to the scale shown under Fig 38
- Fig 28 *Aedes (Aedimorphus) fisheri* Larva, final instar, dorsal view of head drawn from cast skin
- „ 29 *Aedes (Aedimorphus) fisheri* Lateral view of siphon of larva
- „ 30 „ „ „ Two of the smaller pecten teeth
- „ 31 „ „ „ One of the larger pecten teeth
- „ 32 „ „ „ One of the teeth of the comb of the eighth abdominal segment
- „ 33 *Aedes (Aedimorphus) caecus* Lateral plate of the phallosome of the male genitalia, side view, flat preparation
- „ 34 *Aedes (Aedimorphus) vexans* Lateral plate of the phallosome of the male genitalia, side view, flat preparation
- „ 35 *Aedes (Stegomyia) variegatus* Lateral plate of the phallosome of the male genitalia, side view, flat preparation
- „ 36 *Culex fatigans* Outline dorsal view of the terminal abdominal segments of the female
- „ 37 *Aedes (Aedimorphus) vexans* Outline dorsal view of the terminal abdominal segments of the female (c cerci)
- „ 38 *Aedes (Aedimorphus) vexans* Lateral view of the terminal segments of the abdomen of the female, extended after maceration in potash (membrane dotted) (c cerci)
- „ 39 *Aedes (Aedimorphus) stenoetrus* Claw of hind tarsus of male
- „ 40 *Aedes (Aedimorphus) vexans* Claw of hind tarsus of male

PLATE LVI



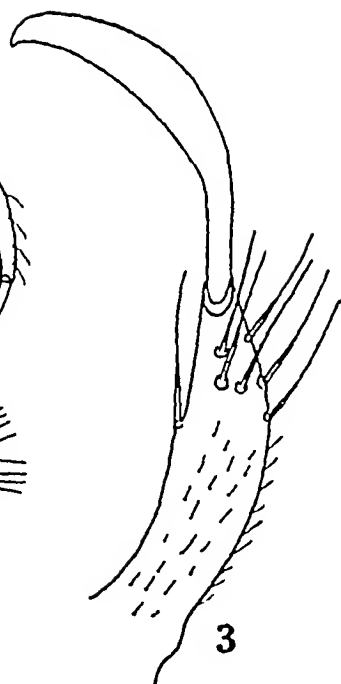
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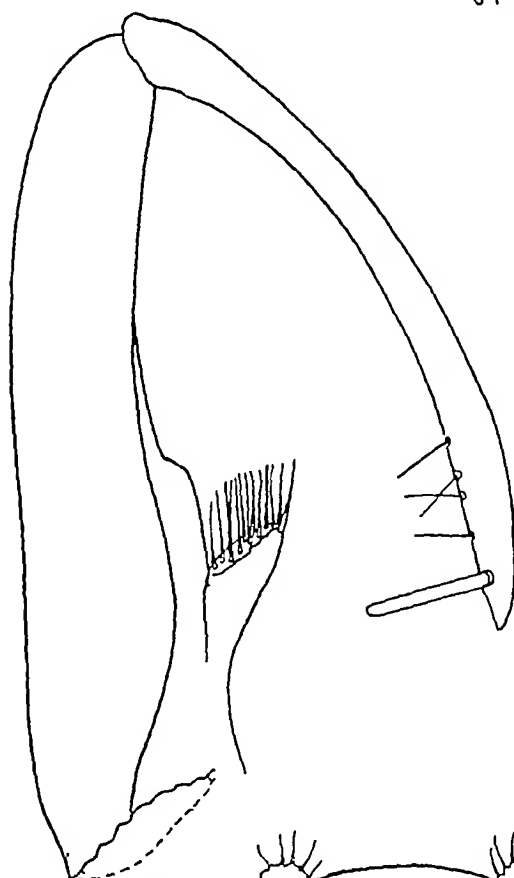
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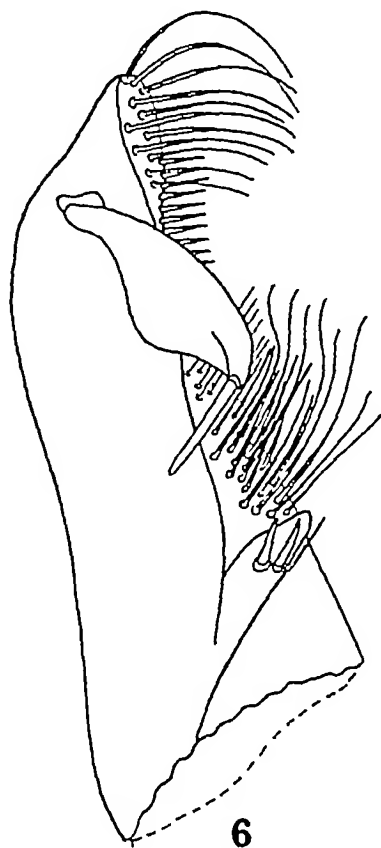


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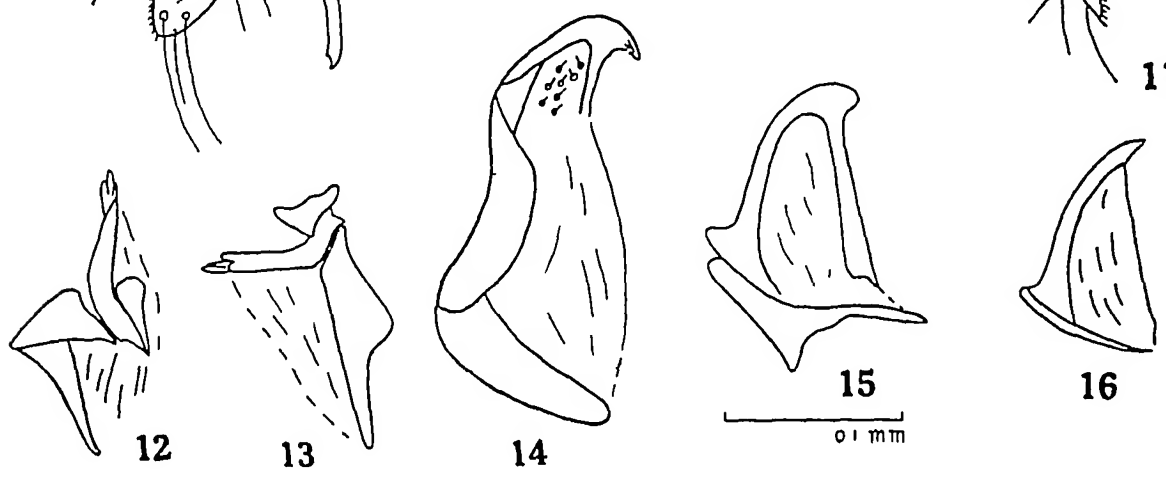
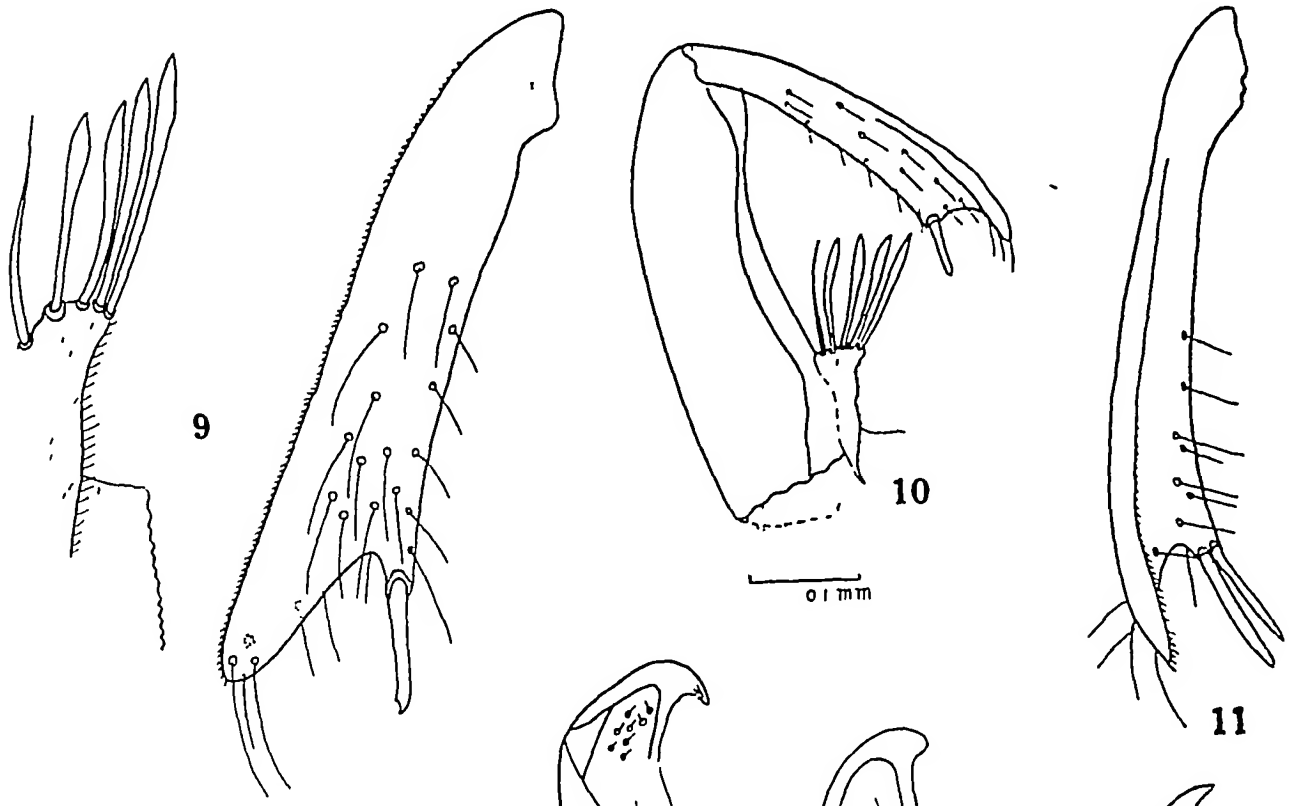
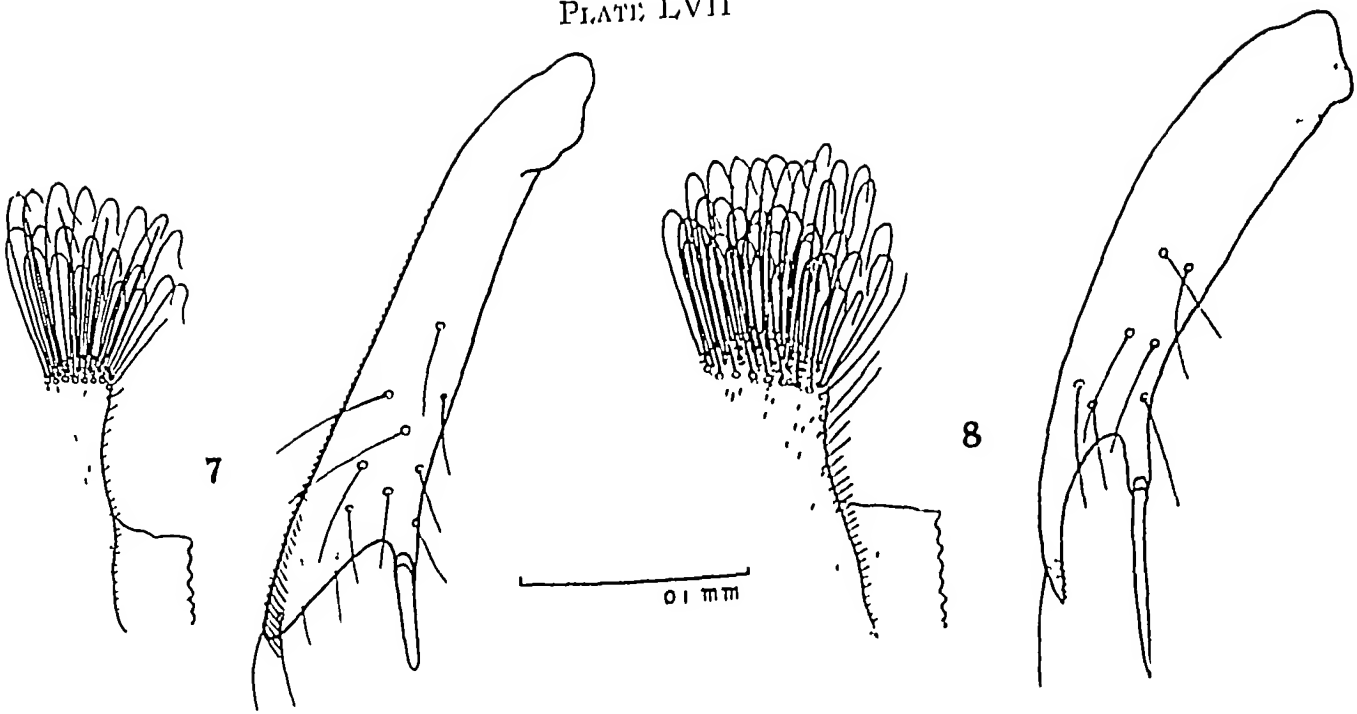


6





PLATE LVII



Anophelines to depend upon the characters of the buccal cavity alone, it would form an entirely separate subgenus

A further and more detailed study of the species examined by these observers and of examples of 34 additional species has brought to light certain other points of interest. The species of the subgenus *Myzomyia* appear to us to fall into three well-defined divisions. The first contains the members of the Group *Neomyzomyia*, with the exception of *A. christyi*, and with the addition of *A. mli*. The second contains all the *Neocellia* Group, the majority of the Group *Myzomyia*, and one of the *Cellias* (*A. pulcherrimus*). The study of a very large series of specimens has convinced us that the differences between these members of the *Myzomyia* Group and the *Neocellias* (with *A. pulcherrimus*) are not sufficient to warrant that they should be placed in different classes as regards the morphology of the buccal cavity. The third division contains the *Pseudomyzomyia* Group, 4 of the *Myzomyia* Group and 2 of the *Cellias*.

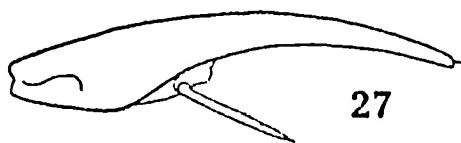
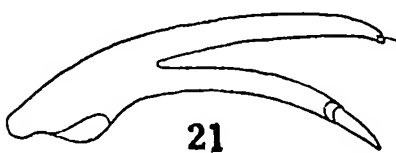
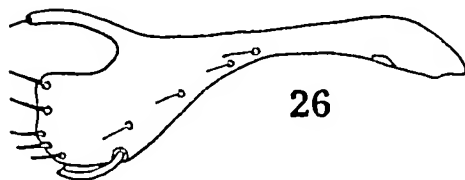
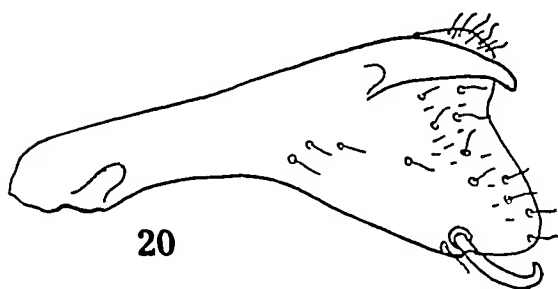
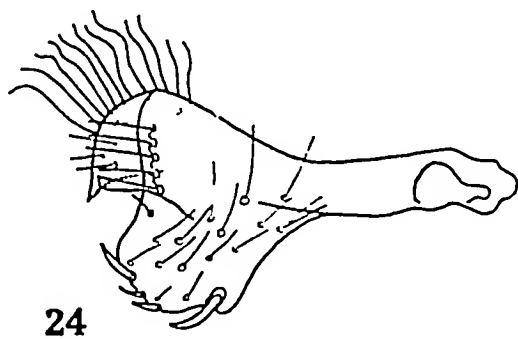
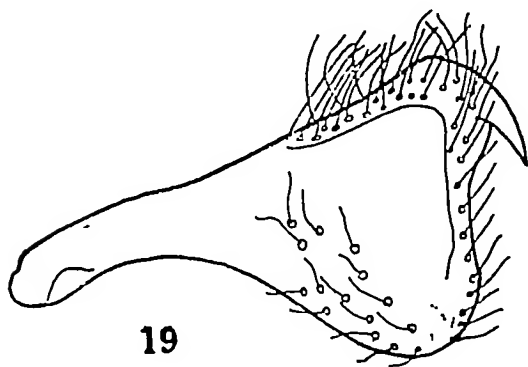
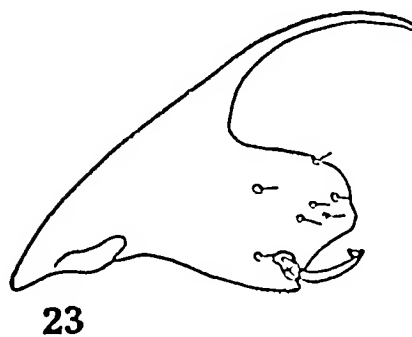
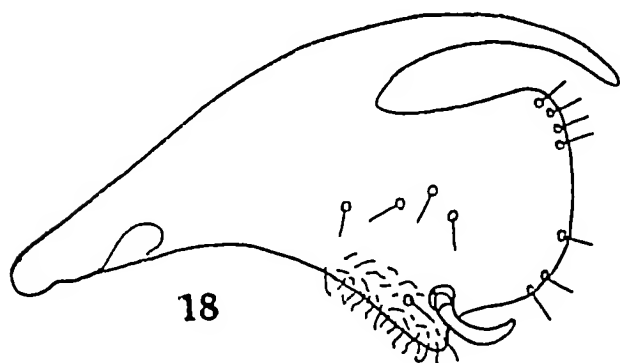
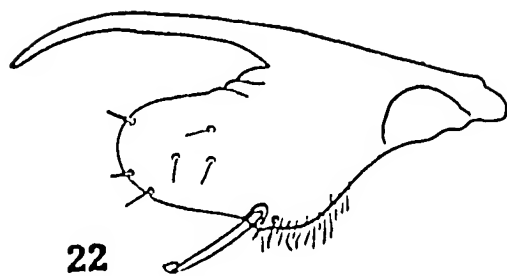
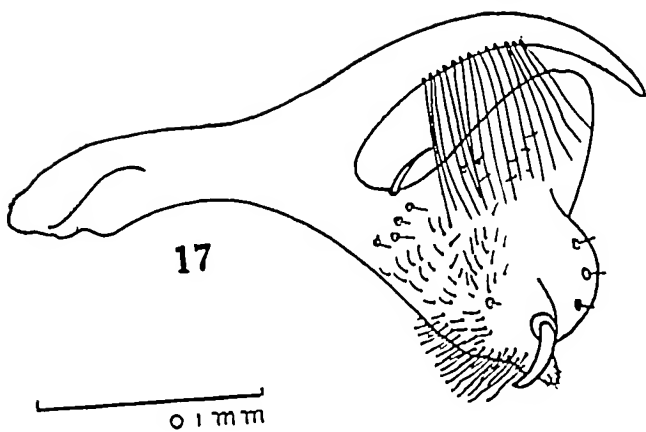
These three divisions appear to us to differ as strikingly from one another as they do from the other subgenera, and it seems preferable to arrange the members of the genus *Anopheles* into 5 Classes as regards the morphology of the buccal cavity. The different characters of these Classes are so pronounced that in the case of the 86 species examined by us it has been found possible at a glance to assign any species to the Class to which it belongs. No intermediate forms have been encountered.

The names of the species we have examined are set out below according to this classification, the generally accepted grouping being indicated beside each species. It is to be noted that no examples of the subgenus *Chagasia* or of the Group *Kerteszia* of the subgenus *Nyssorhynchus* were available for examination.

#### CLASS A Plate LX, (fig 5, Plate LXII, fig 19).

Bucco-pharyngeal armature absent

Name of species	Present Classification	
	Subgenus	Group
<i>A. bironelli</i> Christophers	Bironella	
<i>A. atkenu</i> James	Anopheles	Anopheles
<i>A. algericus</i> Theobald	"	"
<i>A. barberi</i> Coquillett	"	"
<i>A. culiciformis</i> Cogill	"	"
<i>A. bifurcatus</i> Linnæus	"	"
<i>A. plumbeus</i> var <i>barianensis</i> James	"	"
<i>A. maculipennis</i> Meigen	"	"
<i>A. asiaticus</i> Leicester	"	"
<i>A. annandalei</i> Baini Prashad	"	"
<i>A. eiseni</i> Coquillett	"	"
<i>A. lindesayi</i> Giles	"	"
<i>A. gigas</i> var <i>sulensis</i> James	"	"
<i>A. punctipennis</i> Say	"	"



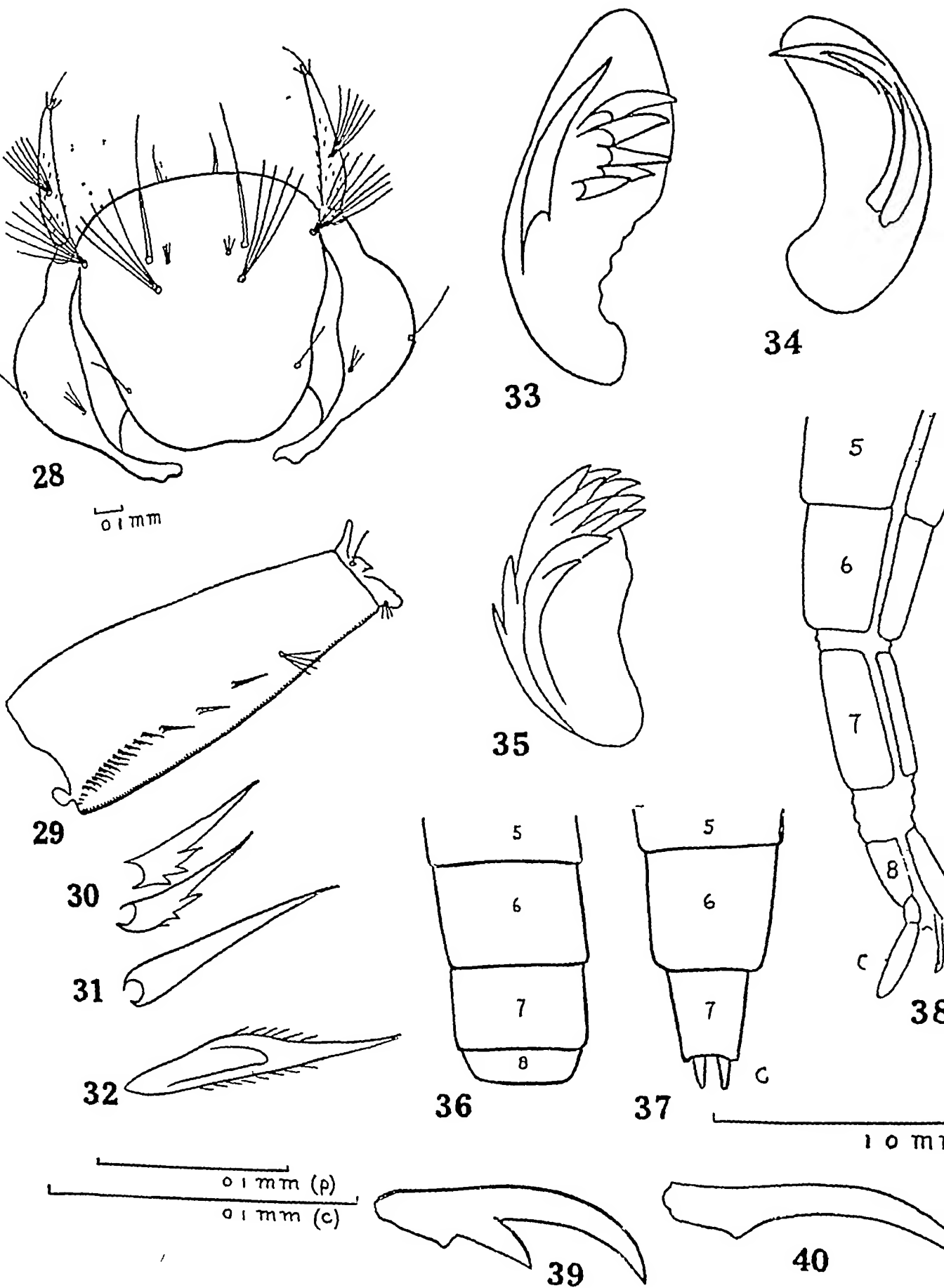
Name of species	Present Classification	
	Subgenus	Group.
<i>A argyritarsis</i> Robineau-Desvoidy	Nyssorhynchus	Nyssorhynchus
<i>A albitarsis</i> var <i>brasiliensis</i> Chagas	"	"
<i>A albimanus</i> Wiedemann	"	"
<i>A tarsimaculatus</i> Goeldi	"	"

## CLASS D (Plate LX, figs 2 to 4, Plate LXI, fig 8)

Bucco-pharyngeal armature of female consists of a double row of teeth without deep-set roots, with 12 to 14 teeth in each row,\* except *A ramsayi*, which has only 8 (Plate LX, fig 3) Line of origin of teeth only moderately curved Bucco-pharyngeal ridges well-developed with simple straight hairs.

Name of species	Present Classification	
	Subgenus	Group
<i>A rhodesiensis</i> Theobald	Myzomyia	Myzomyia
<i>A culicifacies</i> Giles	"	"
<i>A culicifacies</i> var <i>adenensis</i> Christophers	"	"
<i>A sergenti</i> Theobald	"	"
<i>A funestus</i> Giles	"	"
<i>A subumbrosus</i> Theobald ( <i>funestus</i> Giles)	"	"
<i>A funestus</i> var <i>arabicus</i> Christophers and Khazan Chand	"	"
<i>A listoni</i> Liston	"	"
<i>A minimus</i> Theobald	"	"
<i>A acontus</i> Donitz	"	"
<i>A jeyporiensis</i> James	"	"
<i>A jeyporiensis</i> var <i>moghulensis</i> Christophers	"	"
<i>A marshalli</i> Theobald	"	"
<i>A superpictus</i> Grassi	"	"
<i>A transvaalensis</i> Carter	"	"
<i>A rufipes</i> Gough	"	"
<i>A stephensi</i> Liston	"	Neocellia
<i>A fuliginosus</i> Giles	"	"
<i>A philippinensis</i> Ludlow	"	"
<i>A pallidus</i> Theobald	"	"
<i>A maculatus</i> Theobald	"	"
<i>A willmori</i> James	"	"
<i>A theobaldi</i> Giles	"	"
<i>A jamesi</i> Theobald	"	"
<i>A maculipalpis</i> var <i>indiensis</i> Theobald	"	"
<i>A karwari</i> James	"	"
<i>A ramsayi</i> Covell	"	"
<i>A pulcherrimus</i> Theobald	"	Cellia

\* Note—This number does not include the small teeth which can sometimes be seen on the inner aspect of the lateral flanges



and shape of the lateral flanges. The species may be conveniently considered in two groups

(a) Bucco-pharyngeal armature absent

We have examined female specimens of one or more species belonging to the following genera and subgenera—*Megarhinus*, *Uranotaenia*, *Harpagomyia*, *Rachionotomyia*, *Heizmannia*, *Mucidus*, *Armigeres*, *Leicesteria*, *Stegomyia*, *Finlaya*, *Ochlerotatus*, *Banksimella*, *Aedimorphus*, *Aedes*, *Skusea*, *Mansonioides*, *Coquillettia*, *Theobaldia*, *Aedomyia*, and *Orthopodomyia*. In all the species examined the structure of the bucco-pharyngeal junction is very similar and there are no teeth on the posterior end of the ventral plate between the lateral flanges such as are present in our Classes B, C, D, and E of the Anophelines. As will be seen by referring to the drawings reproduced on the accompanying plates the usual number of palatal papillae (Plate LXII, fig 14 pp) is two pairs, but in *Taemorrhynchus* (*Coquillettia*) *crassipes* (van der Wulp) (Fig 27), *Theobaldia* *inveitaemata* (Theo) (Plate LXIII, fig 28), *T. longiarcolata* (Macq), and *Ochlerotatus caspius* (Pallas), there are three pairs. In *Armigeres* and *Leicesteria* the spines on the two pairs of palatal papillae are unusually long (Fig 17). The number of large dorsal papillae (Plate LXII, fig 14 ldp), which are of different structure from the usually more numerous smaller dorsal papillae (Plate LXII, fig 14 sdg) seems to be invariably two on each side of the anterior hard palate (Plate LXII, fig 14 ahg). The number of smaller dorsal papillae, each carrying a rather long hair, varies in the different genera, and, as shown in some of the drawings, the number is not always the same on the two sides of the buccal cavity. This may be due, in some cases, to a slight displacement of the dorsal wall during dissection so that one or more of the papillae may be hidden in a fold at the side. In *Harpagomyia gemirostris* (Leic) (Plate LXVI, fig 40), in which the buccal cavity is very small and narrow, the smaller dorsal papillae appear to be absent, whilst in *Uranotaenia recondita* Edw (Plate LXVI, fig 41) there is only one on each side, in addition to the usual two larger papillae. In the species of the other genera examined they vary in number from two to six on either side of the anterior hard palate. In *Banksimella lineatopennis* (Ludl) (Plate LXII, fig 15) all the dorsal papillae are somewhat posteriorly placed, whilst in *Orthopodomyia*, of which we have examined three species, *anopheloides* (Giles), *flavicosta* Barraud, and *flavithorax* Barraud, one pair of the larger dorsal papillae is placed very far back (Plate LXII, fig 20), as in many species of *Anopheles*, so that the resemblance these mosquitoes bear to certain of the Anophelines is not altogether superficial.

The posterior hard palate (Plate LXII, fig 14 phg) does not show any remarkable modifications, posteriorly it is continued as thin chitin to the junction with the pharynx and this thin plate carries, on the ventral surface (which forms the roof of the buccal cavity in this part), a number of hairs or minute spines. These are well developed in *Mucidus scataphagoides* Theo (Plate LXII, fig 14 pe). The area carrying these spines is homologous with the 'cobblestone area' in *Nyssorhynchus* (Plate LXI, fig 11 cs), and with the 'shagreened area' in *Culex* referred to below. The pigmented area on the posterior hard palate (Plate LXII, fig 14 pa), which is usually well defined in

# THE MORPHOLOGY OF THE BUCCAL CAVITY IN ANOPHELINE AND CULICINE MOSQUITOES

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AND

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[Received for publication, August 16, 1927]

SINTON and COVELL (1927) pointed out that there were certain striking points of difference as regards the morphology of the buccal cavity of the female among the various subgenera of the Anopheline mosquitoes

The present paper is based on the results of a detailed study of examples of 86 species of Anopheline and of 48 species of Culicine mosquitoes. Nothing was previously known regarding the differences in the structure of the buccal cavity in the latter, and our investigations have shown that, as in the case of the Anophelines, there are striking differences in certain groups

The technique employed was the same as that used by Sinton and Covell, i.e., the specimens were macerated in potash, stained with carbol fuchsin, and after dehydration dissected in canada balsam

For a detailed description of the anatomy of the parts of the alimentary canal under consideration the reader is referred to Sinton and Covell's paper. The results of our investigations and the conclusions arrived at from them are set out below

## I ANOPHELINE MOSQUITOES

As the result of the examination of examples of 52 species of Anophelines Sinton and Covell found that as regards the morphology of the buccal cavity the subgenera *Bironella* and *Anopheles* were distinct from all the others in that the females possessed no bucco-pharyngeal armature, that the subgenus *Nyssorhynchus* was markedly different from the other subgenera, and that with regard to the subgenus *Myzomyia* the group *Neomyzomyia* differed so strikingly from the other members of the subgenus that, were the classification of the



we take to be a second row of very fine and minute teeth. In *C fuscocephalus* Theo., and *C gelidus* Theo., the teeth are considerably longer (Plate LXV, fig 37) but the greatest development of the armature in the subgenus *Culex*, as far as our observations go, occurs in *C malayi* (Leic.), *C tenipalpis* Barraud, and *C iphus* Barraud (Plate LXIV, figs 32 Plate LXVII, fig 47). In *C malayi* the teeth are long narrow and very numerous, whilst in *C tenipalpis* they are very closely set and form a curved palisade. It is interesting to note that, as regards the structure of the buccopharyngeal armature, *C malayi* and *C iphus* are distinct from *C khazani*. In all three species the palpi are equally short in both sexes, and in other respects, such as the structure of the male genitalia, they appear to be somewhat closely related. The same remarks apply to *C brevipalpis* and *C tenipalpis*, in which the palpi of the males are about one-half the length of the proboscis and the male genitalia show a general similarity of structure. The two species are however, distinct in the structure of the bucco-pharyngeal armature of the females.

Of the subgenus *Culicatomyia* we have examined females of *viridiventer* Giles, and *pallidothorax* Theo. The armature in both these species is composed of two rows of small sharp teeth numbering from 40 to 50 in each row (Plate LXVI, figs 38 and 39). In the form of the shagreened area and in other details of the structure of the buccal cavity the species belonging to this subgenus resembles those of the *fatigans* group of *Culex* closely.

In *Lophoceratomyia uniformis* Theo., and *L. minor* Leic., the teeth are remarkably developed and the shape and arrangement are noticeably different from those of any other group. As will be seen from Plate LXVII, figs 48 and 50 the line of origin of the teeth forms a continuous curve with the convexity forwards, which is not the case in any of our Groups of *Anopheles* or in *Culex*. The appearance of the shagreened area is shown in Plate LXVII, fig 49. It may be pointed out that the posterior part of the buccal cavity in *Lophoceratomyia*, and in some the smaller species of *Culex*, in which the teeth are numerous, is comparatively large. This will be realised by comparing Plate LXVII, figs 46, 47, and 50, drawn to the same scale, showing this part of the buccal cavity in *Lutzia vorax* (a large *Culex*-like mosquito) and in *C malayi* and *Lophoceratomyia uniformis* (both quite small species).

Our investigations seem to show that, as in the case of the Anophelines, a study of the structure of the buccal cavity in *Culex* will prove of assistance in placing species together in groups. The present observations have necessarily been confined to a limited number of species but the subject appears to warrant further attention.

## SUMMARY

1 The results of the examination of the morphology of the buccal cavity of the female in 86 species of Anopheline, and of 48 species of Culicine mosquitoes are presented in detail.

2 As regards the Anophelines the species may be arranged in five Classes. The members of four of these Classes possess a bucco-pharyngeal armature, this

Name of species	Present Classification	
	Subgenus	Group
<i>A. pseudopunctipennis</i> Theobald	Anopheles	Anopheles
<i>A. crucians</i> Wiedemann	"	"
<i>A. hyrcanus</i> var. <i>ingrimus</i> Giles	"	"
<i>A. albotarqatus</i> Theobald	"	"
<i>A. montanus</i> Stanton and Hacker	"	"
<i>A. separatus</i> Leicester	"	"
<i>A. umbrosus</i> Theobald	"	"
<i>A. novumbrosus</i> Strickland	"	"
<i>A. barbirostris</i> van der Wulp	"	"
<i>A. bancrofti</i> Giles	"	"
<i>A. grabhami</i> Theobald	"	"
<i>A. implevus</i> Theobald	"	Christya
<i>A. vestitipennis</i> Dyar and Knab	"	Arribalzaga
<i>A. pseudomaculipes</i> Chagas	"	"
<i>A. apicimacula</i> Dyar and Knab	"	"
<i>A. punctimacula</i> Dyar and Knab	"	"

## CLASS B Plate LXI, figs 9 and 10)

Bucco-pharyngeal armature of female consists of a single row of large separate pectenate teeth, 8 to 10, in number, except *A. aurcosquamiger* and *A. watsoni*, which have 12 to 14

Name of species	Present Classification	
	Subgenus	Group
<i>A. aurcosquamiger</i> Theobald	Myzomyia	Ncomyomyia
<i>A. kingi</i> Christophers	"	"
<i>A. watsoni</i> Leicester	"	"
<i>A. kochi</i> Donitz	"	"
<i>A. leucosphyrus</i> Donitz	"	"
<i>A. tessellatus</i> Theobald	"	"
<i>A. punctulatus</i> Donitz	"	"
<i>A. punctulatus</i> var. <i>moluccensis</i> Swellengrebel and Swellengrebel	"	"
<i>A. annulipes</i> Walker	"	"
<i>A. marstersi</i> Skuse ( <i>annulipes</i> Walker)	"	"
<i>A. anictus</i> Edwards	"	"
<i>A. aurostris</i> Watson	"	"
<i>A. niki</i> Theobald	"	"
<i>A. umbrosus</i> Theobald of Edwards, 1911 ( <i>niki</i> Theobald)	"	Myzomyia
	"	"

## CLASS C (Plate LXI, figs 11 to 13)

Bucco-pharyngeal armature of female consists of two rows of teeth very markedly recurved, so that their ends are directed forwards (a feature seen in no other group examined) Number of large teeth 8 to 12 Posterior hard palate very highly chitinated, giving a 'cobblestone' appearance.

# EXPLANATION OF PLATE LX

- Fig 1 *Anopheles pharoensis* (Class E) Dorsal view of the posterior end of buccal cavity of female
- „ 2. *A pulcherrimus* (Class D) Dorsal view of the posterior end of buccal cavity of female
- „ 3 *A ramsayi* (Class D) Dorsal view of the posterior end of buccal cavity of female
- „ 4 *A ramsayi* (Class D) Dorsal view of teeth on a larger scale (flattened preparation)
- „ 5 *A implexus* (Class A) Dorsal view of the posterior end of buccal cavity of female

## Lettering —

- php Posterior hard palate
- pa Pigmented area
- vp Ventral papillæ
- lf Lateral flange
- t Bucco-pharyngeal armature
- c 'Cone'
- r 'Rod'
- bpr Bucco-pharyngeal ridges

Figs 1, 2, 3, and 5, drawn to the scale shown under Fig 2

## CLASS E (Plate LX, figs 1, Plate LXI, fig 6, and 7)

Bucco-pharyngeal armature of female consists of a double row of teeth, there being 18 to 26 in each row, except in *A ludlowi* and *A parangensis* which have 14 to 16. The teeth have long deep-set narrow bases, and their line of origin forms a very pronounced curve. Bucco-pharyngeal ridges carry very fine long multiple hairs. Lateral flanges comparatively large.

Name of species	Present Classification	
	Subgenus	Group
<i>A subpictus</i> Grassi	Myzomyia	Pseudomyzomyia
<i>A vagus</i> Donitz	"	"
<i>A ludlowi</i> Theobald	"	"
<i>A ludlowi</i> var <i>sundaica</i> Rodenwaldt	"	"
<i>A parangensis</i> Ludlow	"	"
<i>A gambiae</i> Giles ('costalis')	"	"
<i>A tukkhudi</i> Liston	"	Myzomyia
<i>A flaviceps</i> Edwards	"	"
<i>A multicolor</i> Cambouliu	"	"
<i>A cinereus</i> Theobald	"	"
<i>A christyi</i> Newstead and Carter	"	Neomyzomyia
<i>A pharoensis</i> Theobald	"	Cellia
<i>A squamosus</i> Theobald	"	"

It will be evident from the examination of the results detailed above that in the majority of cases where two species of Anopheline are alike, the structure of the buccal cavity in the female is also very similar. In certain instances, however, it has been found that species which had formerly been thought to be very closely allied differ markedly in this respect.

Thus *A superpictus* (Plate LXI, fig 8) and *A transvaalensis* are in Class D, whilst *A multicolor* (Plate LXI, figs 6 and 7), *A tukkhudi*, *A flaviceps*, and *A cinereus* are in Class E. Again *A pulcherrimus* (Plate LX, fig 2) is in Class D, whereas the other two species of *Cellia* examined *A pharoensis* (Plate LX, fig 1) and *A squamosus* are in Class E. *A nili* (Plate LXI, fig 9), which was formerly placed in Group *Myzomyia*, falls into Class B, whilst *A christyi*, which was placed provisionally among the *Neomyzomyias*, has been shown to belong to Class E.

It is evident that the examination of the buccal cavity may be of great importance in determining the position of a given species, especially in cases where only female specimens are available.

## II CULICINE MOSQUITOES

As regards the Culicines, of the genera so far examined by us only *Lutzia* and *Culex*, including the subgenera *Culicomyia* and *Lophocerotomyia*, possess a bucco-pharyngeal armature in the female. In the other genera, in which the armature is absent, the structure of the buccal cavity is similar to the species falling within our Class A of Anopheles, except in certain details of number and arrangement of the palatal and dorsal papillae, and in the comparative size

# EXPLANATION OF PLATE LXI

- Fig 6 *Anopheles multicolor* (Class E) Dorsal view of the posterior end of the buccal cavity of female
- „ 7 *Anopheles multicolor* (Class E) Dorsal view of a few teeth on a larger scale
- „ 8 *A. superpictus* (Class D) Dorsal view of the posterior end of the buccal cavity of female
- „ 9 *A. nini* (Class B) Dorsal view of the posterior end of the buccal cavity of female
- „ 10 *A. kochi* (Class B) A few of the teeth (flattened preparation)
- „ 11 *A. albimanus* (Class C) Dorsal view of the posterior end of the buccal cavity of female
- „ 12 *A. tarsimaculatus* (Class C) Posterior portion of ventral plate of buccal cavity of female The lateral flanges have been removed, and three of the large teeth broken off
- „ 13 *A. albimanus* (Class C) Lateral view of the posterior part of the buccal cavity and commencement of pharynx of female (lateral flanges not shown The same specimen from which Fig 11 was drawn)

## Lettering —

- php Posterior hard palate
- pa Pigmented area
- vp Ventral papillæ
- lf Lateral flange
- t Bucco-pharyngeal armature
- c 'Cone'
- r 'Rod'
- ph Pharynx
- cs 'Cobblestone area'
- bc Buccal cavity

Figs 6, 8, 9, and 11 drawn to the scale shown under Fig 6

„ 7, 10, and 12 drawn to the scale shown under Fig 7

the various groups of the Anophelines, is not always well marked in the species under discussion. The number of ventral papillæ (Plate LXII, fig 14 vp) seems to be always two pairs situated near the posterior end of the ventral plate.

(b) Bucco-pharyngeal armature present

This group includes only the genera *Lutzia* and *Culex*, with the subgenera *Culicomyia* and *Lophoceratomyia*.

The teeth forming the armature in the buccal cavity of the females of *Lutzia*, of many species of *Culex*, and of *Culicomyia*, are smaller and arise in a different position from those present in our Groups B, C, D, and E, of the Anophelines (compare Plate LXII, figs 6, 8, 9 and 11 with Plate LXV, figs 34, (Plate LXV., fig 39, Plate LXVII, fig 46). In some species of *Culex* and in the subgenus *Lophoceratomyia*, the teeth are longer and very numerous, and number in some species more than 50 (Plate LXIV, figs 32, Plate LXVII, figs 47, and 50). As in the Anophelines no teeth are present in the buccal cavity of the males (compare Plate LXVI, figs 42 and 43).

Fig 29 shows a lateral view of the buccal cavity and part of the pharynx of the female of *Culex tenuipalpis* Barraud, and Fig 30 shows a dorsal view of the buccal cavity of the same species on the same scale, but somewhat flattened and with the clypeus removed. These two drawings are given as a guide to the morphology of the various parts referred to in the following notes.

The shape of the anterior hard palate, and the arrangement and number of the palatal, dorsal, and ventral papillæ, are very similar to those occurring in the species mentioned under group (a), and call for no special comment, except that in *Lutzia* (Plate LXVII, fig 45) the four palatal papillæ are placed closed together as is the case in most species of the Anophelines.

In all the species examined the posterior hard palate carries on the dorsal surface a minute prominence, the dorsal apodeme (Plate LXIV, fig 29 da), which appears to be connected with the pigmented area (Plate LXV, fig 35 pa). Posterior to this the dorsal plate becomes thinner, but is covered on the ventral surface with a large number of small processes giving it a shagreened appearance, which we have called the 'shagreened area' (Plate LXV, fig 35 sa), in the middle line these processes are usually more pronounced and form a tongue-like projection (Plate LXV, fig 35). This area forms the roof of the buccal cavity and seen from above it lies immediately dorsal to the teeth so that unless it is removed the latter cannot be clearly seen. For this reason the posterior hard palate and the pigmented area are not shown in many of the figures, as the dorsal plate had to be removed to expose the teeth.

In *Lutzia fuscana* (Wied), *L. vorax* Edw, and a number of species of *Culex* including *bitaeniorhynchus* Giles, *fatigans* Wied, *tipuliformis* Theo, *theileri* Theo, and *pipiens* L., the teeth are comparatively short and blunt. In some of the more heavily stained preparations the tips of the teeth appear to be thin and spoon-shaped. We have endeavoured to indicate this in Plate LXII, fig 46.

*C. vishnu* Theo, *C. mimeticus* Noe, *C. brevipalpis* (Giles), and *C. khazani* Edw, possess a very similar armature to that of the *fatigans* group, but the teeth appear to be more pointed, and in some of our preparations there is what

## EXPLANATION OF PLATE LXII

Fig 14	<i>Mucidus scataphagoides</i>	Dorsal view of buccal cavity of female
„ 15	<i>Banksimella lineatopennis</i>	„
„ 16	<i>Heizmannia himalayana</i>	„
„ 17	<i>Armigeres obturbans</i>	„
„ 18	<i>Stegomyia albopicta</i>	„
„ 19	<i>Anopheles bifurcatus</i> (Class A)	„
„ 20	<i>Orthopodomyia flavicosta</i>	„
„ 21	<i>Anopheles culicifacies</i> (Class D)	The same of the male

Lettering —

ahp	Anterior hard palate
pp	Palatal papillæ
ldp	Larger dorsal papillæ
sdp	Smaller dorsal papillæ
clt	Torn edge of clypeus
php	Posterior hard palate
pa	Pigmented area
pe	Posterior thin extension of posterior hard palate carrying minute spines or hairs on the ventral surface
vp	Ventral papillæ
lf	Lateral flange

All the Figs are drawn to the scale shown under Fig 15

feature being absent in the remaining one. The differences in the structure of the buccal cavity in the five Classes are so marked that it has been found possible at a glance to place any species examined in the Class to which it belongs. In certain cases it has been shown that species which have been thought to be very closely allied differ remarkably as regards the morphology of the buccal cavity.

3 As regards the Culicines, of the genera examined, only *Lutzia* and *Culex*, including the subgenera *Culicomyia* and *Lophoceratomyia* possess a bucco-pharyngeal armature. The structure of this differs very markedly in different groups. As in the case of the Anophelines it has been shown that certain species which in other respects appear to be allied differ strikingly as regards the morphology of the buccal cavity.

4 It is concluded that although the study of the morphology of the buccal cavity of mosquitoes is generally of little help in the differentiation of closely allied species, yet it is of great value in indicating the group to which a given species belongs and may prove of importance in cases where only the female of a species is available for examination.

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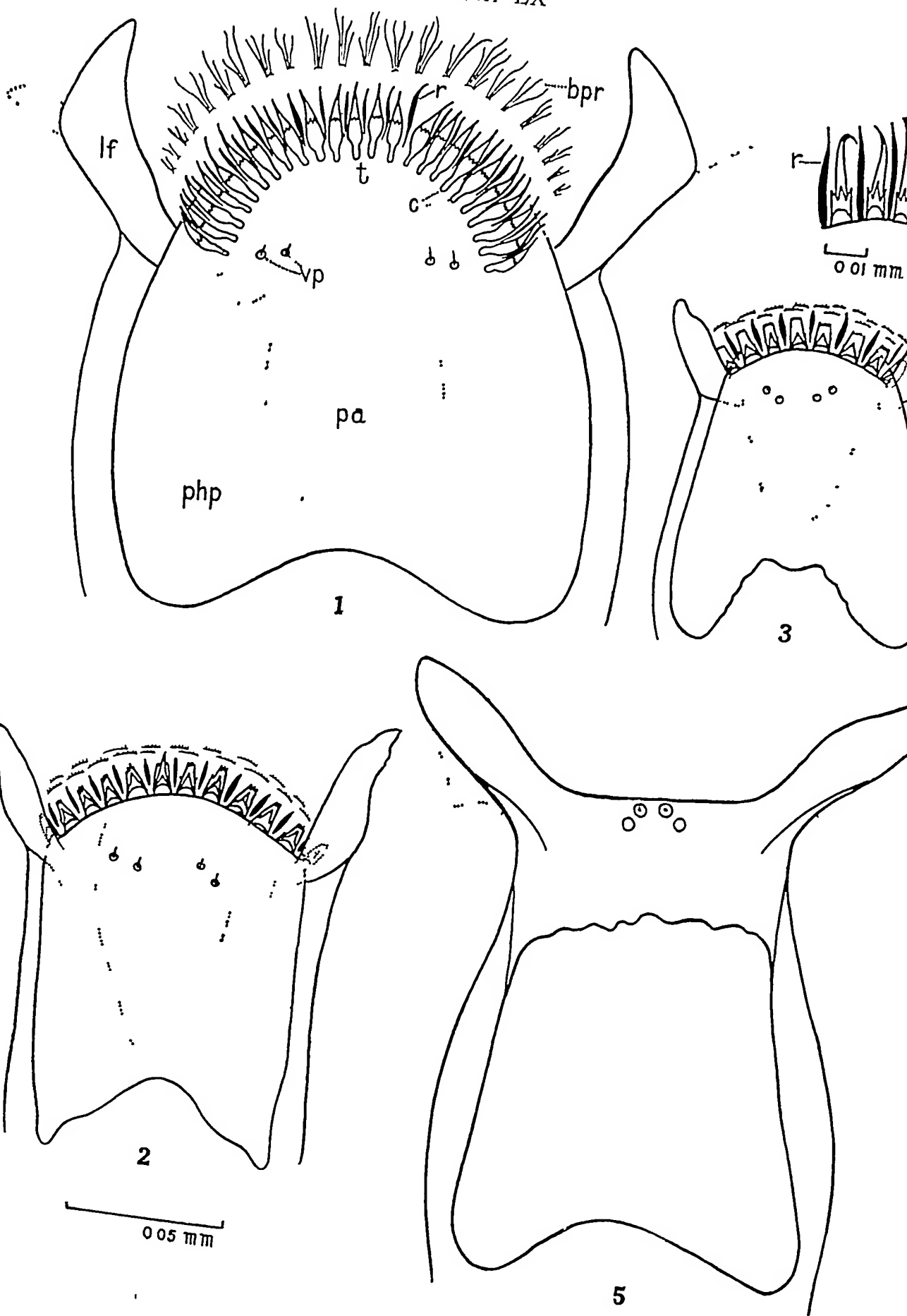
# EXPLANATION OF PLATE LXIII

Fig 22	<i>Megarhinus splendens</i>	Dorsal view of the buccal cavity of female
„ 23	<i>Skusea microptera</i>	„
„ 24	<i>Aedimorphus veians</i>	„
„ 25	<i>Aedomysia venustipes</i>	„
„ 26	<i>Mansomoides uniformis</i>	„
„ 27	<i>Taeniorhynchus (Coquillettidia) crassipes</i>	„
„ 28	<i>Theobaldia mventaemata</i>	„

## Lettering —

ahp	Anterior hard palate
pp	Palatal papillæ
ldp	Larger dorsal papillæ
sdp	Smaller dorsal papillæ
clt	Torn edge of clypeus
php	Posterior hard palate
pa	Pigmented area
pe	Thin extension of posterior hard palate
vp	Ventral papillæ
lf	Lateral flange

All the figures are drawn to the scale shown under Fig 28



# EXPLANATION OF PLATE LXIV

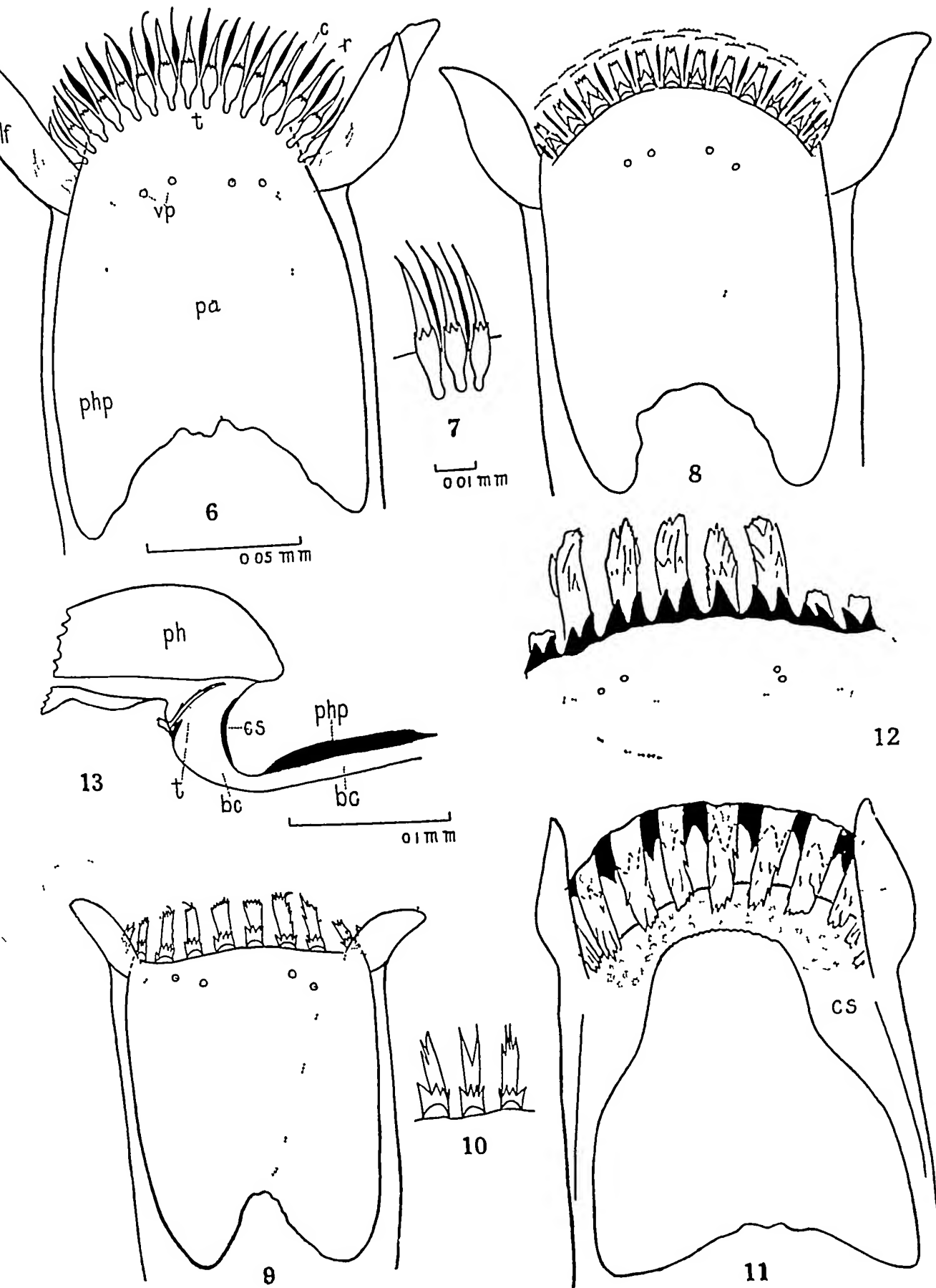
- Fig 29 *Culex tenuipalpis* Lateral view of the buccal cavity and part of the pharynx of the female
- „ 30 *Culex tenuipalpis* Dorsal view of the buccal cavity (posterior part of dorsal plate indicated in outline only)
- „ 31 *Culex tenuipalpis* Dorsal view of the posterior hard palate and shagreened area, which lie over the teeth shown in Fig 32
- „ 32 *Culex tenuipalpis* Dorsal view of the posterior end of the ventral plate showing the teeth

## Lettering —

- bp Commencement of the biting mouth parts
- sp Salivary pump
- dp Dorsal papillæ
- bc Buccal cavity
- php Posterior hard palate
- vp Ventral papillæ
- t Teeth forming bucco-pharyngeal armature
- bpj Bucco-pharyngeal junction
- ph Pharynx
- sa Shagreened area
- lf Lateral flange
- da Dorsal apodeme
- ahp Anterior hard palate
- cl Clypeus
- clt Torn edge of clypeus

Figs 29 and 30 drawn to the scale shown over Fig 29

„ 31 and 32 drawn to the scale shown under Fig 31



# EXPLANATION OF PLATE LXV

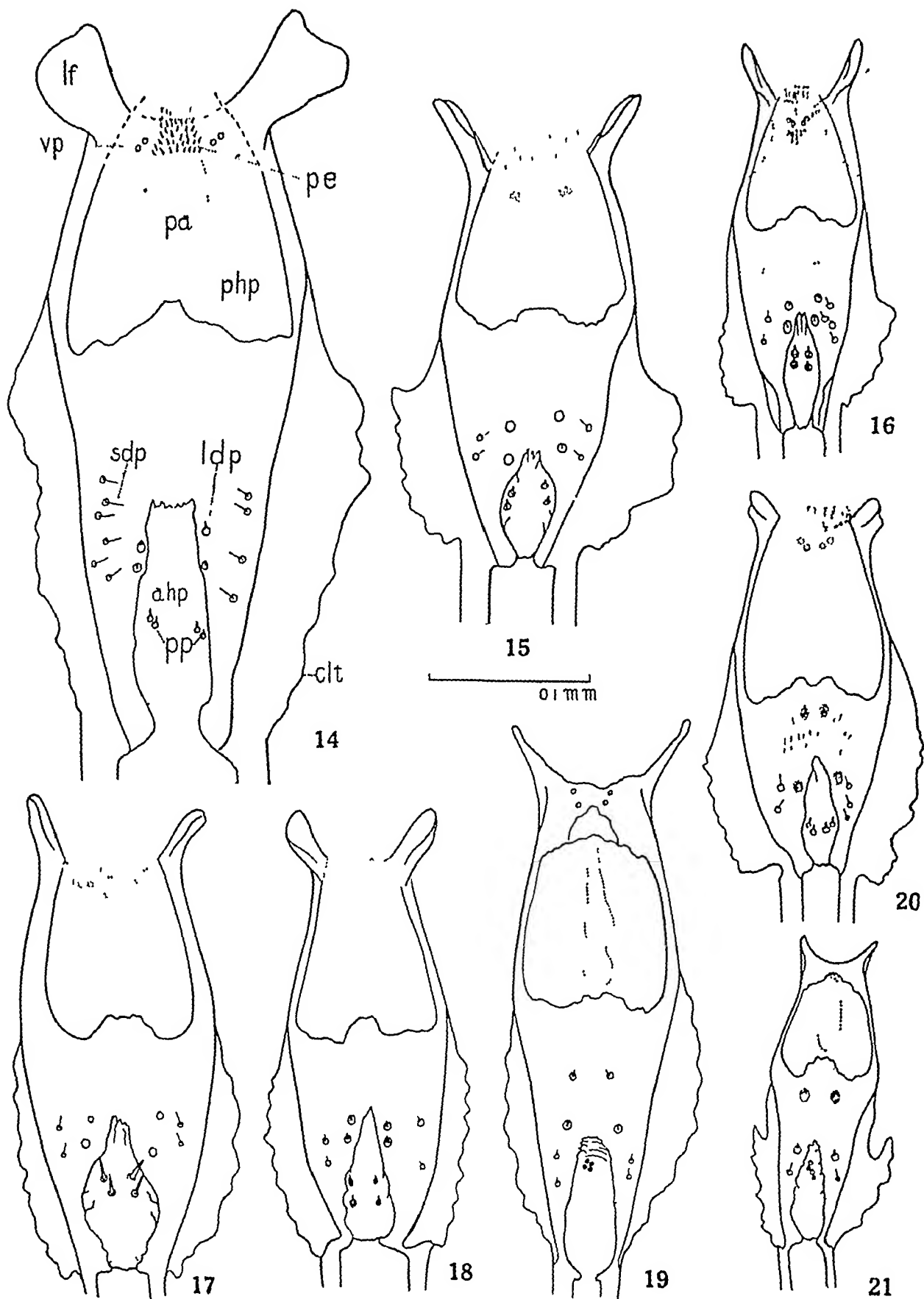
- Fig 33 *Culex bitaeniorhynchus* Dorsal view of the buccal cavity of female  
(posterior hard palate and shagreened area not shown)
- „ 34 *Culex bitaeniorhynchus* Dorsal view of the posterior end of the  
ventral plate showing teeth
- „ 35 *Culex bitaeniorhynchus* Dorsal view of the posterior hard palate and  
shagreened area
- „ 36 *Culex fuscocephalus* Dorsal view of the posterior end of dorsal  
plate of buccal cavity of female
- „ 37 *Culex fuscocephalus* Dorsal view of the posterior end of ventral  
plate of buccal cavity (dorsal plate seen in Fig 36 removed)

## Lettering —

- ahp Anterior hard palate
- pp Palatal papillæ
- ldp Larger dorsal papillæ
- sdp Smaller dorsal papillæ
- clt Torn edge of clypeus
- vp Ventral papillæ
- t Teeth forming bucco-pharyngeal armature
- lf Lateral flange
- sa Shagreened area of dorsal plate
- da Dorsal apodeme
- php Posterior hard palate
- pa Pigmented area

Fig 33 drawn to the scale shown at the side, Figs 34 to 37 to scale shown  
under Fig 36

PLATE LXII



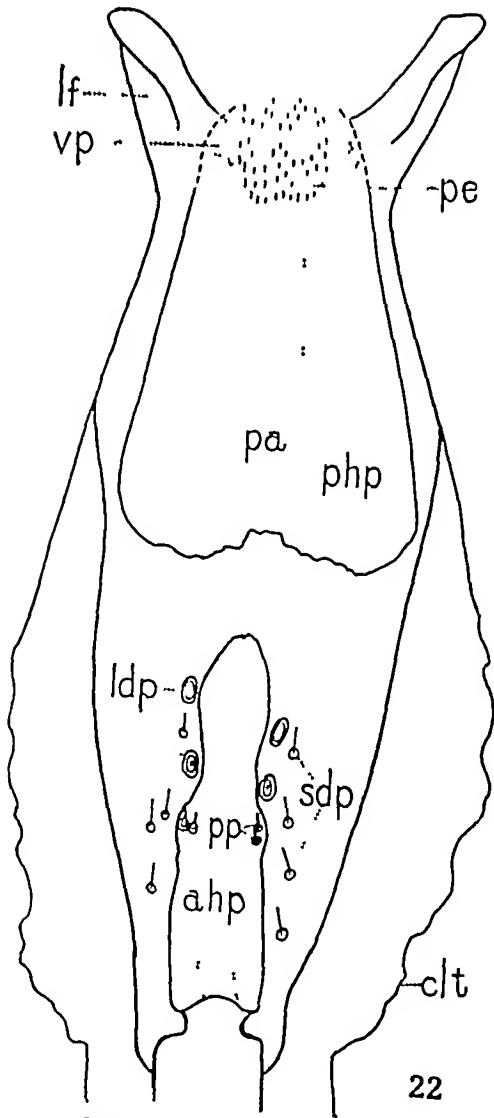
# EXPLANATION OF PLATE LXVI

- Fig 38 *Culicomyia pallidothorax* Dorsal view of buccal cavity of female  
(posterior part of dorsal plate not shown)
- „ 39 *Culicomyia viridiventer* Dorsal view of posterior part of ventral  
plate of buccal cavity of female
- „ 40 *Harpagomyia genurostris* Dorsal view of buccal cavity of female
- „ 41 *Uranotaenia recondita* Dorsal view of buccal cavity of female
- „ 42 *Culex fatigans* The same of the male
- „ 43 *Culex fatigans* The same of the female (posterior part of the dorsal  
plate not shown)
- „ 44 *Rachnotomyia aranoides* Dorsal view of buccal cavity of female

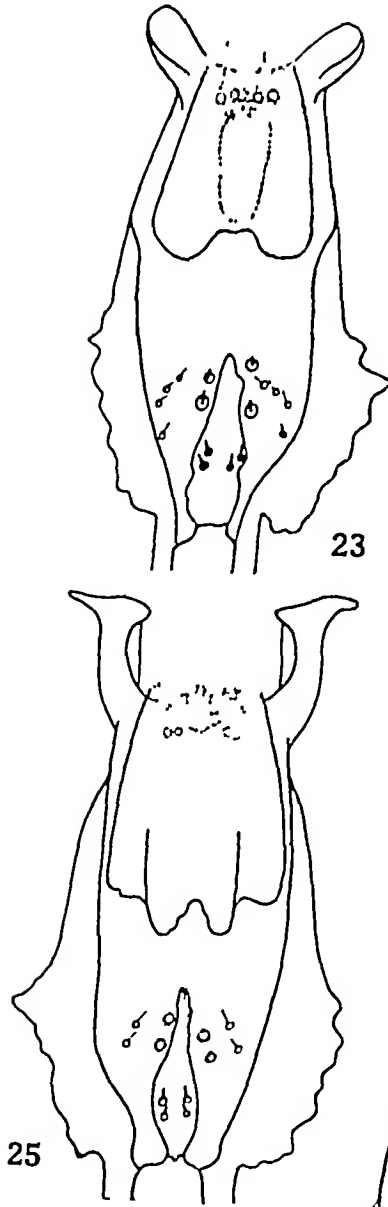
## Lettering —

- ahp Anterior hard palate  
pp Palatal papillæ  
ldp Larger dorsal papillæ  
sdp Smaller dorsal papillæ  
clt Torn edge of clypeus  
vp Ventral papillæ  
lf Lateral flange  
t Teeth forming bucco-pharyngeal armature

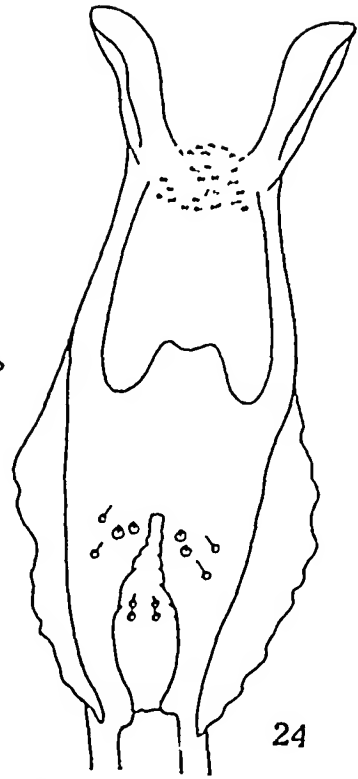
Figs 38 and 40 to 44 drawn to the scale shown under Fig 38



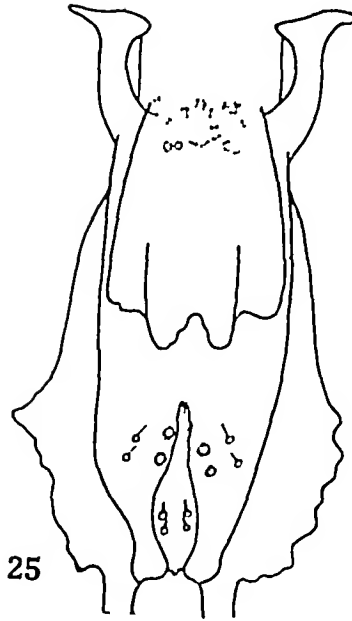
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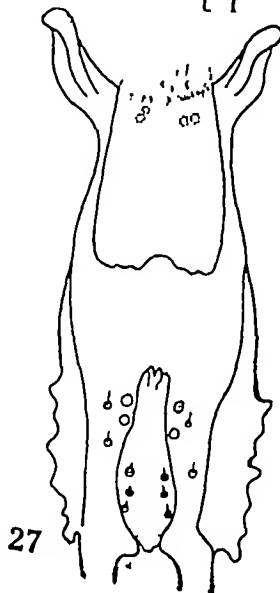
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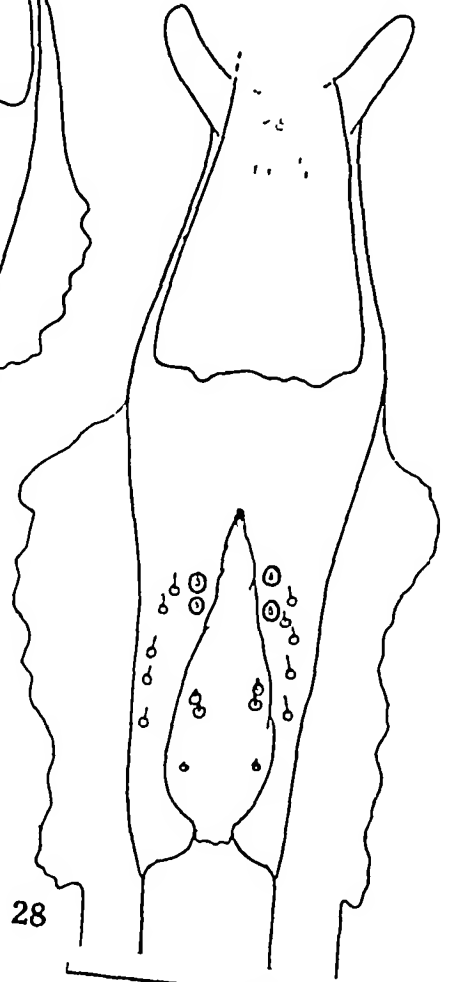
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26



27



28

0.1 mm



## EXPLANATION OF PLATE LXVII

- Fig 45 *Lutzia fuscana* Dorsal view of buccal cavity of female (posterior part of dorsal plate not shown)
- „ 46 *Lutzia vorax* Dorsal view of posterior end of ventral plate of buccal cavity of female, showing teeth
- „ 47 *Culex malayi* Dorsal view of posterior end of ventral plate of buccal cavity of female, showing teeth
- „ 48 *Lophoceratomyia uniformis* Dorsal view of buccal cavity of female (posterior part of dorsal plate not shown)
- „ 49 *Lophoceratomyia uniformis* Dorsal view of posterior part of dorsal plate of buccal cavity of female
- „ 50 *Lophoceratomyia uniformis* Dorsal view of posterior part of ventral plate of buccal cavity of female

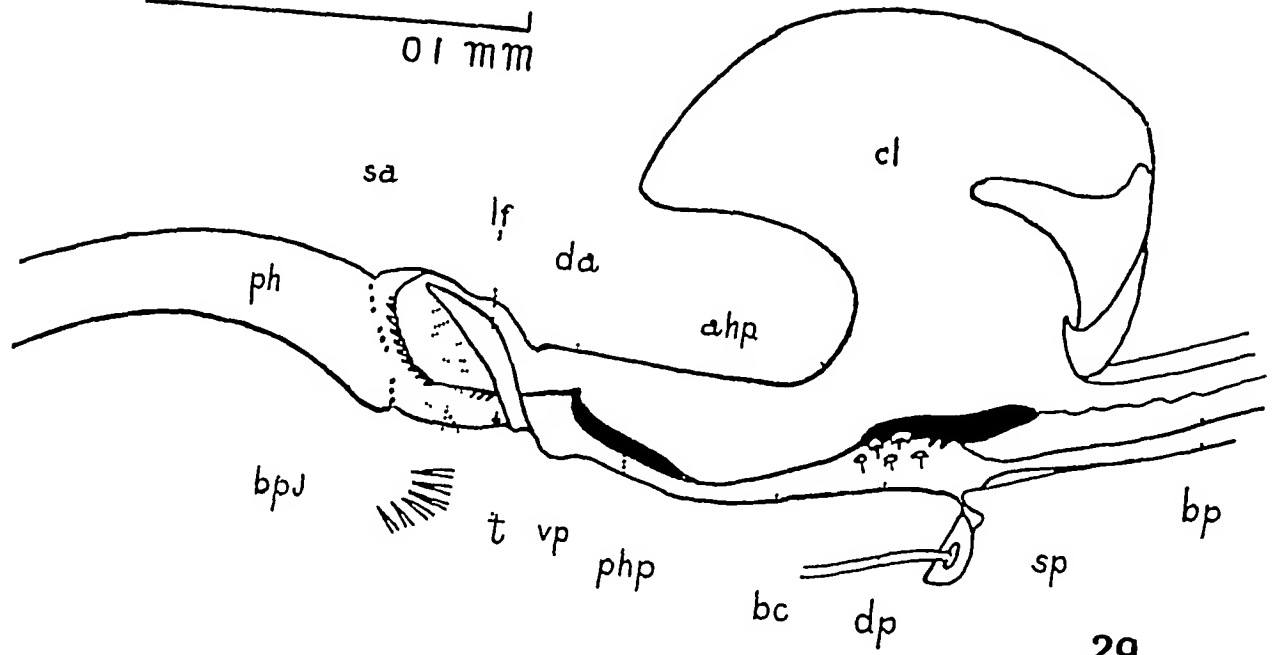
### Lettering —

- ahp Anterior hard palate
- pp Palatal papillæ
- ldp Larger dorsal papillæ
- sdp Smaller dorsal papillæ
- clt Torn edge of clypeus
- vp Ventral papillæ
- t Teeth forming bucco-pharyngeal armature
- lf Lateral flange

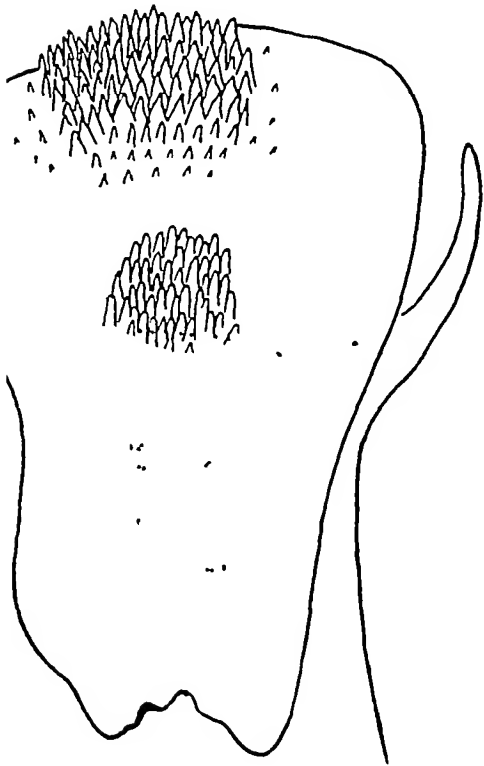
Figs 45, 48, and 49 drawn to the scale shown under Fig 48

„ 46, 47, and 50 drawn to the scale shown under Fig 50

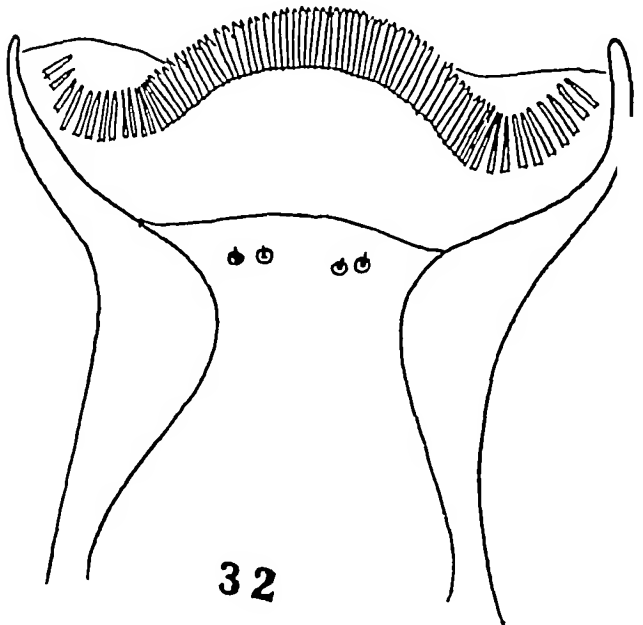
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29

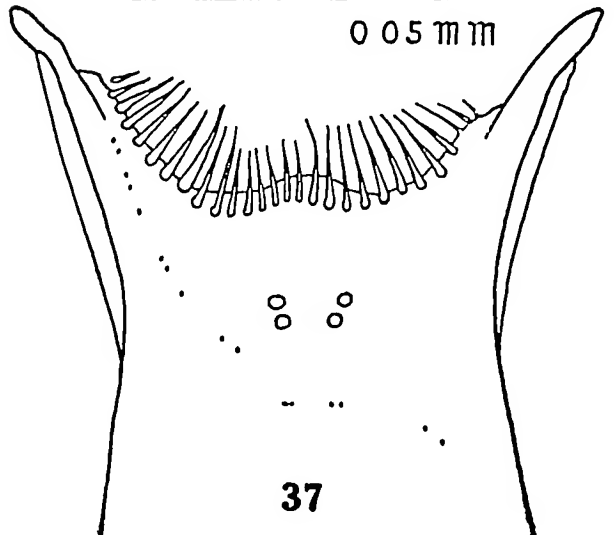
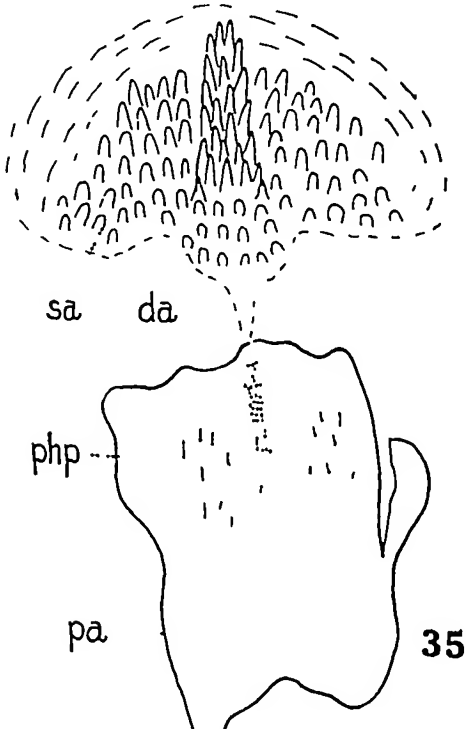
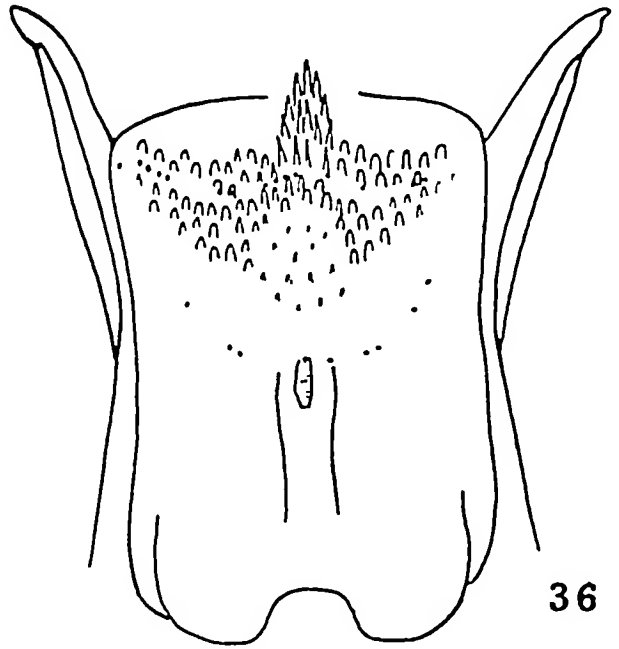
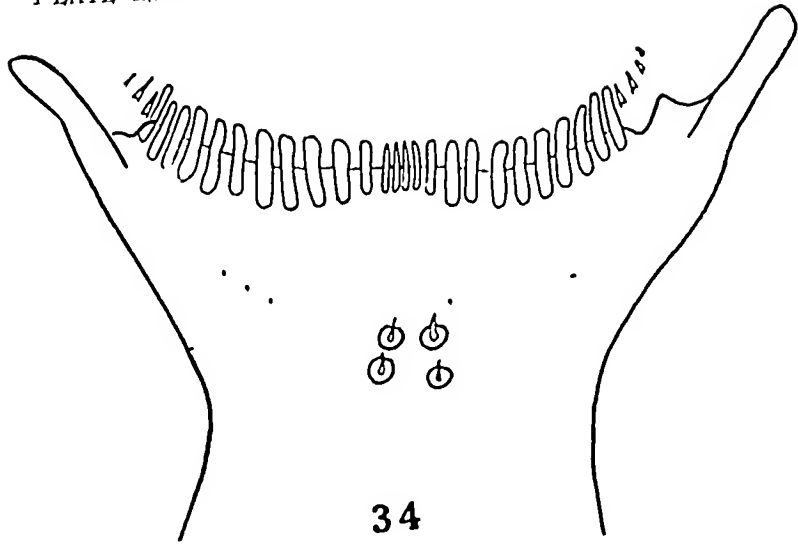
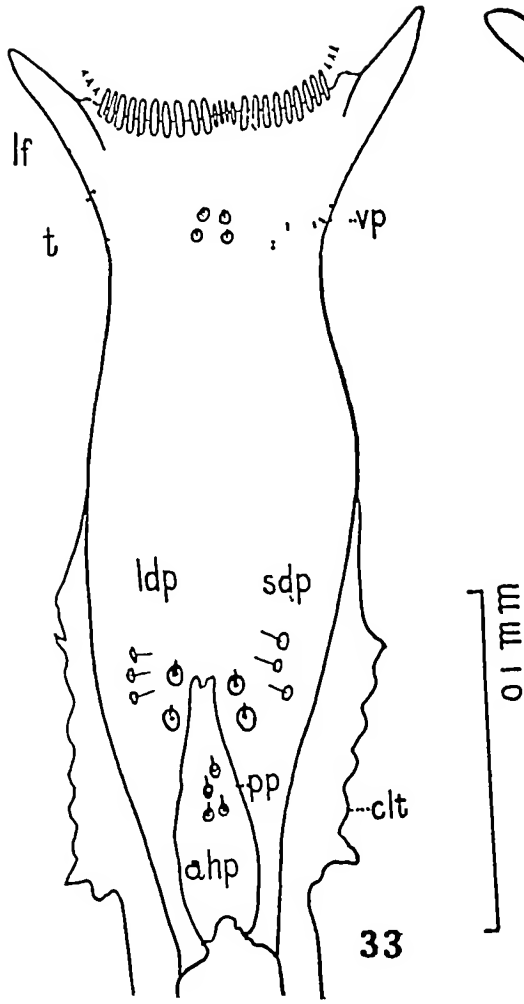


0.05 mm



32





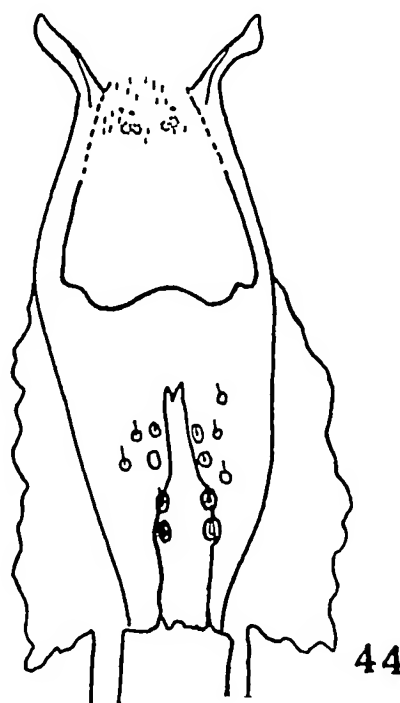
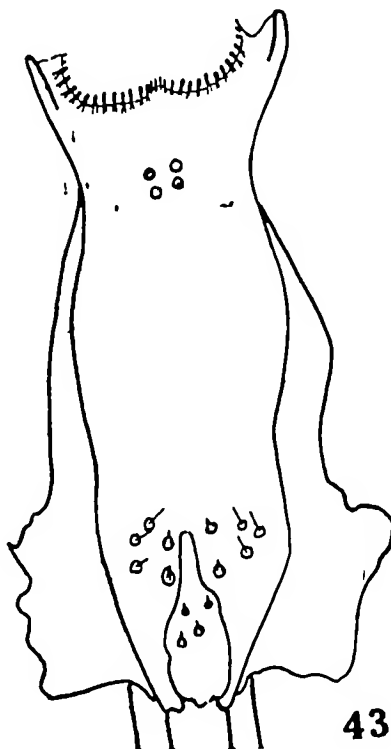
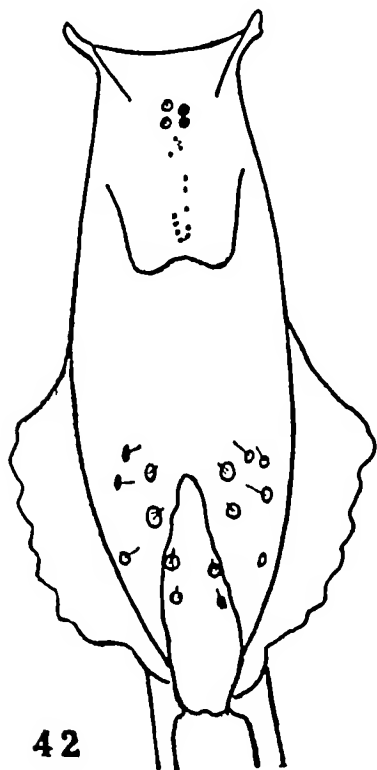
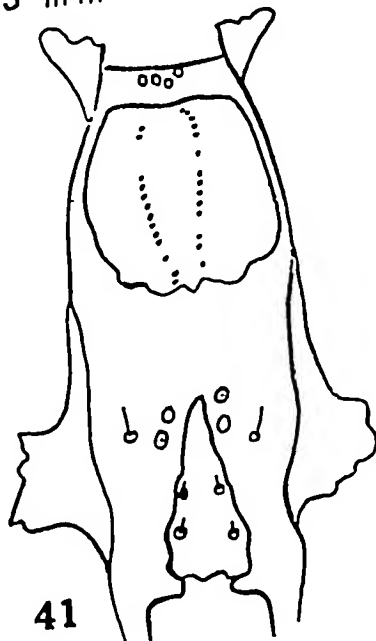
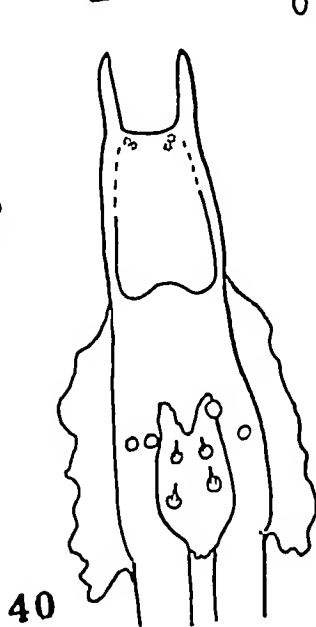
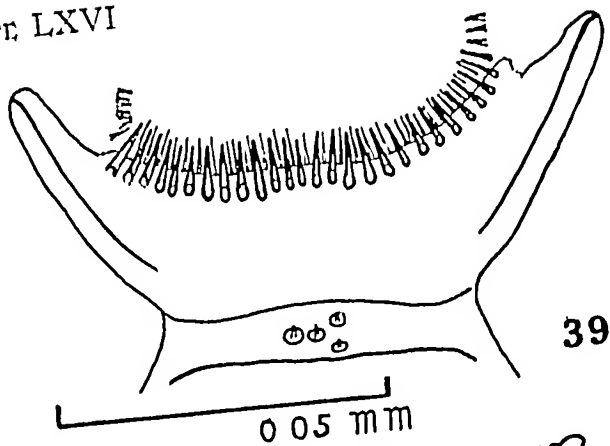
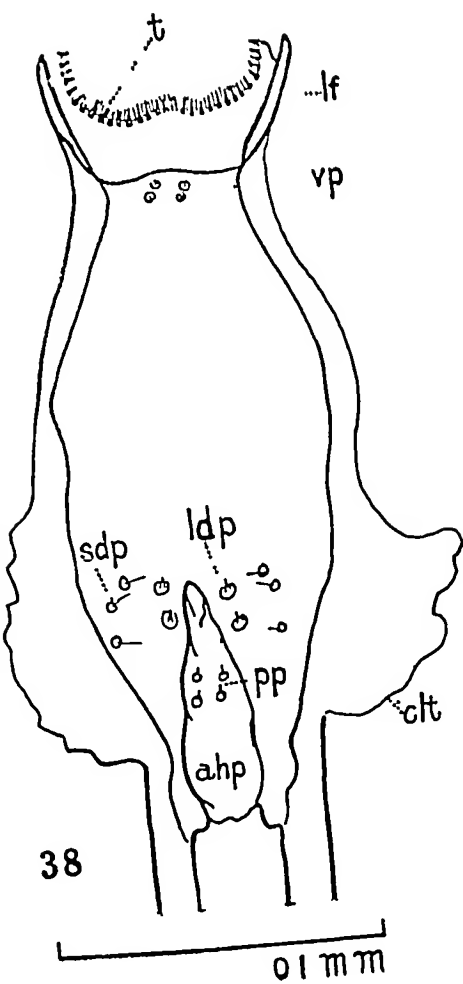
The natural vacillations are apparent

Only recently Landsteiner (1927) has drawn attention to 'the frequent occurrence of positive reacting sera in stock rabbits' Uchida (1927) quoting Kolmer and Trist has mentioned that 'heating the serums of normal rabbits, dogs and mules at 55°C for 15 to 30 minutes greatly increases their property of yielding non-specific complement fixation reactions with various antigens of tissue extract, bacteria, etc'

In view of the paucity of detail as to the precautions and controls in the work on experimental syphilis, *the changes in quality and quantity of the natural reactions with mere passage of time*, and the increasing popularity of the flocculation tests for detecting syphilis the writer thinks that the observations would stand repetition, hence the communication

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| KAHN, R L (1925)                        | Serum Diagnosis of Syphilis by Precipitation, p 147<br>Williams and Wilkins Company, Baltimore   |
| KAHN, R L (1926)                        | <i>Jour Amer Med Assocn</i> , December, p 2092   |
| LANDSTEINER, K. and SCHER, J V D (1927) | <i>Jour Exper Med</i> , March, p 465   |
| UCHIDA, S (1927)                        | <i>Jour Inf Dis</i> , May, p 605   |



ordinary technique of Wassermann reaction (probably, ordinary as opposed to Kolmer's technique-writer) Our actual results with Kahn test in leprosy though yet few, are proving satisfactory' (Lloyd, Muir and Mitra, 1927), again that leprosy serums deviate the complement with a syphilitic antigen almost as well as syphilitic serums The Kolmer modification and Kahn precipitation test seem more advantageous as, according to some workers, they are absolutely negative in leprosy (Gomes as abstracted by a 'regular correspondent' of *Jour A M A*, 1927), again that 'the authors, therefore, conclude that the Kahn precipitation test is negative in uncomplicated cases of leprosy, even when lepra reaction is present, and so is preferable to the Wassermann test in detection of treponematosus infection in lepers' (Pineda, E V and Pineda, E R as abstracted in the *Trop Dis Bull*, and in the *Ind Med Gazette*, 1927), and again that 'the Kahn reaction has been found on investigation, alongside the Wassermann reaction to be exceedingly suitable for testing the presence of syphilis in leprosy' (Report of the Governing Body of the Indian Research Fund Association for the year 1925-26)

In the beginning of 1927, at Kasauli, the writer started employing Kahn test, alongside Wassermann reaction, in the routine for detection of syphilis and in specially selected cases The following two tables are of interest —

TABLE I

## 112 Lepers.

WASSERMANN.		KAHN.			MICROKAHN		
		Positive.	Doubtful.	Negative.	Positive	Doubtful.	Negative.
Positive	37	21	6	10	23	4	10
Doubtful	9	4	1	4	3	1	5
Negative	66	0	6	60	0	8	58
112							

The number represents the total population of Sabathu Leper Asylum (Simla Hills) between 13th August, 1927 and 1st September, 1927 No attempt at classification, with regard to type and duration, has been made No untainted children have been included Previous anti-syphilitic treatment has been excluded

TABLE II

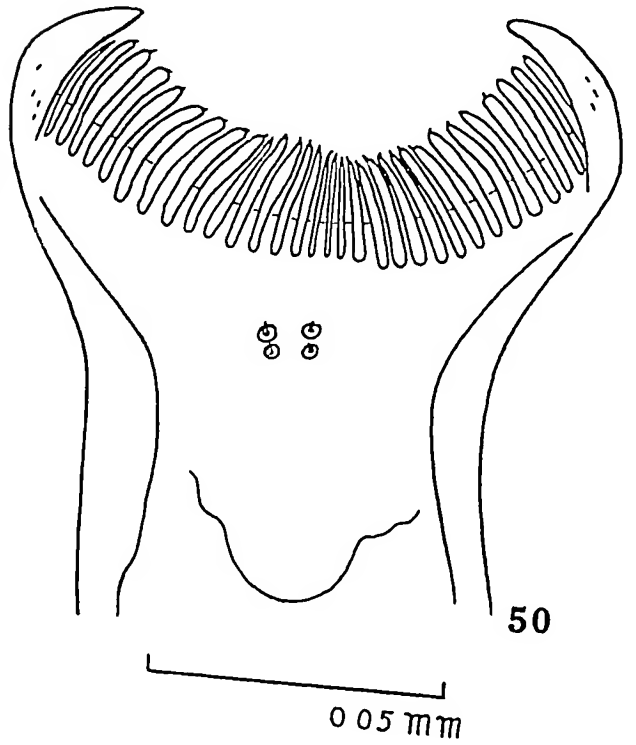
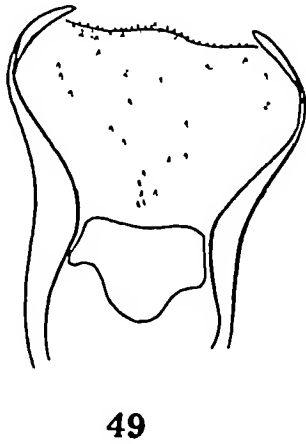
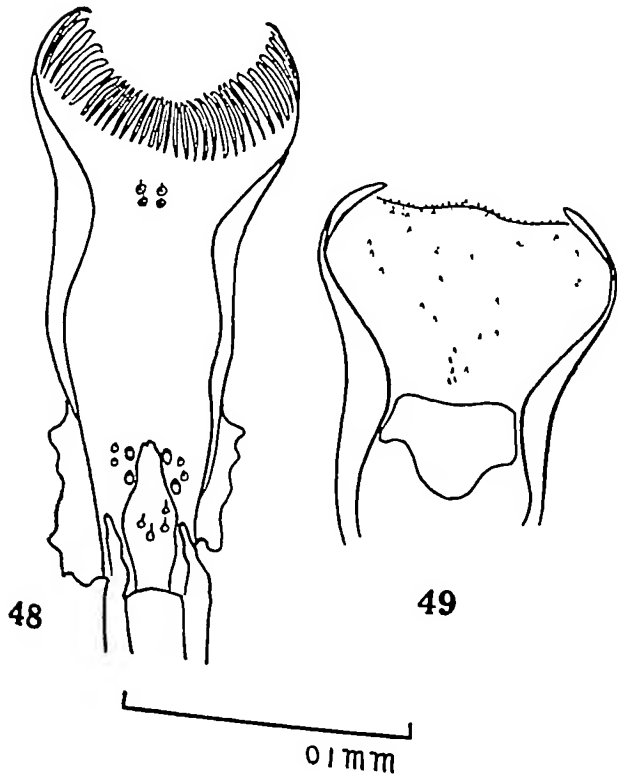
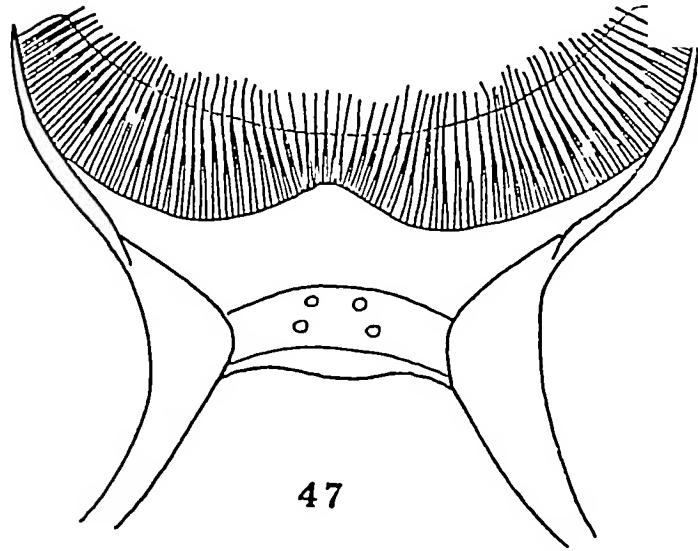
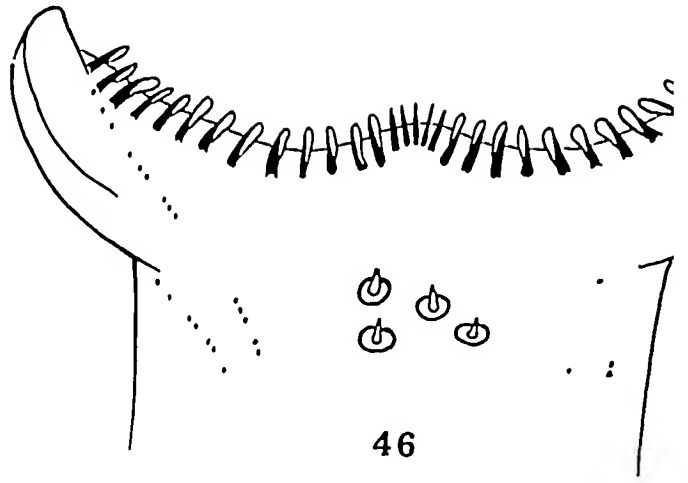
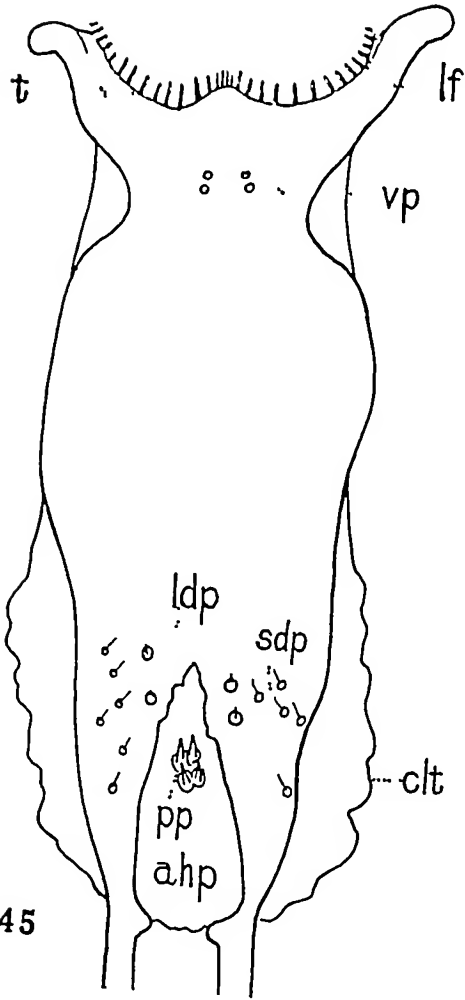
## 112 Syphilitic Suspects

WASSERMANN.		KAHN.		MICROKAHN		
		Positive.	Doubtful Negative.	Positive	Doubtful.	Negative.
Positive	38	Not done.		19	2	17
Doubtful	5	At Kasauli serum is usually		1	0	4
Negative	69	received in small quantities.		3	4	62
112						

Wassermann { Positive = + + +, + +, +  
 Doubtful = +  
 Negative = -

Kahn\* { Positive = + + + +, + + +, + +  
 Doubtful = +, ±  
 Negative = -

\* As\* followed by Kahn (1925), p 168, in comparing his results with Wassermann The system followed by the Medical Department of U S Navy is different It gives more details





- KAHN (1926) — *Jour Amer Med Assoc*, December, p 2092.  
 Medical Research Committee, 1918 The Wassermann Test  
 LLOYD, MUIR and MITRA (1927) *Ind Jour Med Res*, January, p 667  
 GOMES (1927) *Jour Amer Med Assoc*, July, p 45  
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 Report of the Governing Body of the Indian Research Fund Association, for the year 1925-26-27, p 2  
 GREENBAUM (1926) *Jour Amer Med Assoc*, April, p 1273  
 GREENBAUM (1927) *Jour Amer Med Assoc*, April, p 1289  
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# APROPOS OF STUDIES IN SYPHILIS IN RABBITS RABBITS NATURALLY POSITIVE TO WASSERMANN AND KAHN

BY

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[Received for publication, August 25, 1927]

Five normal rabbits were marked and isolated The following table gives the reaction of their sera

Rabbits	27th April, 1927		4th May, 1927		10th May, 1927		4th July, 1927		8th August, 1927	
	W R *	Kahn †	W R	Kahn	W R	Kahn	W R	Kahn	W. R	Kahn
No 1	±	Not done	+±	++++	±	++	±	+++	Died	
No 2	+		±	+	—	++	—	—	±	++
No 3	—		+±	++	—	++	+±	+++	+	++
No 4	—		—	++	—	++	±	+	—	—
No 5	—		—	++	—	++	±	++	—	++

\* W R The technique followed was that of Method IV recommended by the British Medical Research Committee with the following differences —

- 1 A small volume procedure was followed
- 2 An extra tube with 8 M H D of complement was introduced A reaction in this tube was termed a +++ reaction.
- 3 An extra serum control with 1½ M H D of complement was introduced This control guarded against a false + or ± reaction

† Kahn A one-tube Micro-Kahn test was done as recommended by its author (1925 and 1926) The quantities used were —

Antigen dilution  
Serum  
Saline

01 c.c.  
1 "  
2 "

The antigen was kindly supplied by Dr Kahn himself

were obtained by both methods. It would appear, however, that strictly comparable conditions were not observed by these workers in the study of the two methods. We have found that variations in different factors, viz., temperature, concentration of the respective reagents, and the time of contact, affect in an appreciable degree the respective values obtained by the two methods.

To verify Munson and Tolman's results we made the conditions strictly uniform in both cases. A stock solution of iodine was used in glacial acetic acid of exactly decinormal strength. From this was prepared Hanus' solution by adding bromine, and Wijs' by passing dry chlorine gas through it until the thiosulphate titre of the original solution was exactly doubled in both instances. The concentration of both solutions was thus equal. Strictly comparable conditions were observed throughout in the matter of bottles, quality of oil, carbon tetrachloride, time, temperature, and titration with sodium thiosulphate. The following are the results —

TABLE I

*Comparative values for iodine number by Wijs' and Hanus' method*

Names of Glycerides	Wijs' method	Hanus' method
1 Mustard oil	100.2	100.0
2 Ghee	36.4	36.8
3 Ghee	31.97	32.4
4 Ghee	36.56	37.0
5. Ghee	33.9	34.0
6. Mustard oil	107.5	107.2
7. Mustard oil	96.6	95.5
8. Mustard oil	97.5	97.8
9. Linseed oil	176.7	175.3
10 Coconut oil	9.2	8.15
11. Ground-nut oil	93.8	93.4
12 Sesame oil	108.5	108.1

From these results we note that, in nearly all, identical values are obtained by both the methods, any divergency between the two values being within the limits of experimental error. The differences in the values, however, in the case of numbers 7, 9, and 10 are outside experimental error. In these the Wijs' value is distinctly higher than the Hanus'. In No. 7 for instance, if we apply the legal standards strictly the sample would pass by Wijs' method and fail by Hanus'. The sample as a matter of fact was found to be adulterated with sesame oil.

# KAHN,\* MICROKAHN† AND WASSERMANN‡ IN LEPROSY

BY

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[Received for publication, October 1, 1927]

It has been recently reported that 'we have to recognise the possibility that ordinary accurate Wassermann technique which has been quantitatively adjusted to detect syphilis, may show in leprosy, in addition to a large number of positives which are undoubtedly due to syphilis, a certain number of positives which are not so due, and which result from the fact that the technical process employed may not be sufficiently attuned to the special case of leprosy. We term this the experimental factor.

While we are strengthened then in the conclusion formerly reached that every case of leprosy before being given antileprotic treatment should be serologically examined for the presence of syphilis, for the present we are inclined to recommend Kahn test for the purpose rather than the

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\* As detailed by its author in his book (1925)

The same method is recommended in the 'Technique of Kahn test for Syphilis employed in the Medical Department, U S Navy,' a document received from Dr Kahn on 24th April, 1927

The antigen was kindly supplied by Dr Kahn himself. The titre was stated on the bottle. This antigen is now known to keep its potency almost indefinitely [Correspondence, *J A M A* (1927), Kendrick and Jenks (1926)]

† As recommended by its author, in his book (1925) p 147, and in a later communication (1926) a one-tube test was done. The quantities used were —

Antigen dilution	01 c.c.
Serum	1 c.c.
Saline	2 c.c.

‡ Method IV of the (British) Medical Research Committee Report with the following difference —

- (1) A small volume procedure was followed, using quill tubes, stirring rods and Wright's pipettes
- (2) An extra tube with 8 M H D of complement was put up. A positive reaction in this tube was termed a +++ reaction
- (3) An extra serum control with  $1\frac{1}{2}$  M H D of complement was put up. This control guarded against a reading of false positive resulting from an anti-complementary titre of the serum lying between  $\frac{1}{2}$  and 2 M H D of the complement.

iodine, and to pass washed and dried chlorine gas until the titration number of the original solution is doubled' Later he qualifies this by saying 'It is preferable to have a slight excess of iodine, an excess of chlorine is to be avoided as some substitution is liable to occur' Similarly to prepare Hanus' (1901) solution it is stated 'Dissolve 13.2 gms of pure iodine in 1 litre of glacial acetic acid and to the cold solution add 3 c.c. of bromine or sufficient practically to double the halogen content *but with the iodine slightly in excess*' Here, however, no reason is ascribed for the 'slight excess of iodine' as in Wijs' solution

To the analyst this 'slight excess of iodine' should be well defined and definite, as it is not evident what the effect would be on the iodine values if iodine is or is not added in excess, either of which may happen To elucidate this we prepared two lots of five solutions, one set according to Wijs' and the other to Hanus' In each set two solutions were made of higher strength and two of lower strength than double the titration number of the original decinormal iodine solution in glacial acetic acid by adding excess or less of chlorine or bromine according as required Pure ground-nut oil was selected for study Nearly the same amount of oil was taken in each case The reaction time allowed was  $2\frac{1}{2}$  hours at room temperature The results are shown below From these results graphs were constructed on the same scale showing the variations of the iodine values in each case with the variation in the strength of the blank solution

TABLE III

*Iodine values of ground-nut oil from the Hanus' solutions of varied strengths*  
(a)

Weight of oil in grammes	Blank in c.c. of thiosulphate	No. of c.c. of thio sulphate for the surplus iodine.	Iodine value
'2160	53.25	38.55	88.0
'2180	43.20	28.05	90.0
2026	40.35	26.10	90.9
'2122	33.90	19.45	88.2
2216	31.10	16.60	84.7

(b)

*Iodine values of ground-nut oil from the Wijs' solutions of varied strengths*

2183	57.10	38.75	107.3
2189	45.25	29.25	93.3
'2133	40.00	24.95	90.5
2178	34.45	19.40	88.2
2188	29.45	14.45	87.4

Cases have been taken serially from the daily record book They represent the type of cases in whom syphilis has been suspected clinically

The tables are more or less alike The following conclusions emerge —

1 The Wassermann positive rate of the lepers compares so well with the Wassermann positive rate of the syphilitic suspects that one could include the lepers in the syphilitic suspects

2 A one-tube Microkahn is a good representative of the full-size Kahn

3 Kahn has reduced the positive rate in both the series The reduction, which is actually less in the case of the lepers, does not appear to depend on the elimination of a 'lepra reaction'

4 If the above assumption is correct, with Kahn there is a risk of missing some syphilitic cases in leprosy This risk, along with a risk of an opposite nature (which the writer does not consider so important), in general work, has been recognised as an 'error of basing serum diagnosis of syphilis on the Kahn reaction alone' (Greenbaum 1926, 1927)

The writer is aware of a point of difference in his work In making a comparison lepers have generally been compared with apparently healthy latent cases of syphilis with a Wassermann positive rate varying between 22 per cent (Iyengar, 1919) and 15—20 per cent (Lloyd, Muir and Mitra, 1927) The writer has compared them with syphilitic suspects The Wassermann positive rate in leprosy, as given by different workers or even the same workers at different times, has differed considerably and does not call for remarks

The assumption that the series in Table II was composed of syphilitic suspects is based on the clinical notes accompanying the hospital specimens These specimens were tested free The hospitals concerned, however, did not practise testing of blood as a routine in every case In non-hospital cases a fee of Rs 32 charged was a sufficient guarantee of the fact that the tests were not done as a part of a general annual overhaul Most of these cases too were accompanied by clinical notes

## SUMMARY

1 In comparing 112 lepers with an equal number of non-lepers in whom syphilis was suspected (syphilitic suspects), Wassermann figures were almost identical

2 Kahn reduced the positive rate in both the series The reduction was less in the case of the lepers

3 There was no evidence to show that the lower positive rate with Kahn was due to an elimination of the 'lepra reaction'

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Serum Diagnosis of Syphilis by Precipitation  
Baltimore, Williams & Wilkins Co  
*Jour Amer Med Assoc*, May, 1927, p 1663  
*Jour Lab and Clin Med*, January

Correspondence

KENDRICK and JENKS (1926)

brings in an element of doubt. In case of both *Wij's* and Hanus' it would likely give a lower value than the 'standard value' otherwise obtained. The higher value obtained in case of the *Wij's* when its titre exceeds the 'standard stage' is due to the formation of  $\text{ICl}_3$ , whose halogens are in a labile condition and able to produce substitution as well as addition in the glyceride molecule. But in the case of Hanus' no substitution takes place at all, as then the full amount of halogen necessary for saturation of the unsaturated linkages is not available owing to the formation of  $\text{IBr}_3$  and other derivatives whose halogens are in stable combination. The lower value obtained with both solutions when the titre of the final solution is less than that at the 'standard stage' is, of course, due to the smaller amount of iodine monochloride or monobromide being formed than is necessary for full saturation of the glycerides.

*Method of preparation of 'standard' Wij's solution*—Hanus' solution is very convenient to prepare, as it requires only the addition of 2.9 c.c. of pure bromine to a litre of N/10 iodine solution. *Wij's*, however, is more difficult as it requires an apparatus for the generation of dry chlorine gas and also occasional pipetting of the pungent solution for titration with thiosulphate solution so as to enable the analyst to see when the 'standard stage' is just reached. The process is thus not only tedious and irksome but involves the loss of some original solution. Lewkowitsch says 'a little experience will readily show when this point is reached, as a very distinct change of colour takes place when all the iodine has been converted in iodine monochloride'. This change of colour though noticeable at this stage is rather difficult to spot unless the analyst has had some considerable experience of the colour required. By repeated trials we find that an aqueous solution of 1.6 per cent of potash bichromate possesses the same tint as the 'standard' *Wij's* solution, when viewed through transmitted light in two narrow test tubes or preferably cylinders of uniform size (as in the colorimetric determination of pH). The bichromate solution will keep its tint indefinitely if kept in an amber-coloured bottle. In preparing the solution the analyst has only to pour out the solution, while the chlorine is passing through it, into a narrow cylinder and compare its tint with the standard tint in a similar cylinder. The darker tint means that more chlorine is required and the lighter tint means more chlorine has been passed than is necessary. In the latter case this can be rectified by adding more of the original N/10 iodine solution until the 'standard' tint is reached. This method is simple and saves much trouble in pipetting and titration, and is more certain.

*The effect of time on the iodine values*—Leach (1920) says that only half an hour is required for full saturation of oil with iodine from Hanus' solution. With *Wij's* solution also it is stated that semi-drying oils, e.g., mustard oil, poppyseed oil, etc., require the same length of time. Tolman and Munson however recommend for the Hanus' solution one hour's standing in all cases especially with oils having iodine values as high as 100. In view of these statements it was desirable to study the two methods so far as the time effect was concerned. Mustard oil was taken as it is the only edible oil whose iodine value is included in the standards of the Bengal Food Adulteration Act. In view also of economy of time it is important that the analyst should know the minimum

# COMPARATIVE OBSERVATIONS ON THE WIJS' AND HANUS' METHODS OF DETERMINING THE IODINE VALUES OF OILS

BY

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THE iodine value is an invaluable guide to the detection of sophistication in edible oils and fats. In the case of mustard oil it has been adopted as one of the legal standards by the Bengal Food Adulteration Act which lays down limits of 96 to 108. Although several new methods have within the last few years been described, none of them possess any advantages over Wijs' and Hanus' methods. These methods have of late replaced altogether the pioneer method of Hubl on account of the stability of their reagents, the comparative ease with which the reagents can be prepared and the smaller interval of time required by them for the saturation by iodine of the glycerides. Wijs' method is generally employed in Europe whereas Hanus' is the official method of the Association of Agricultural Chemists in America. It is therefore important from the legal point of view that the values obtained by the two methods should if possible agree.

The most recent comparative study of the different methods was made in 1923 by S. Schmidt Nielson who pronounces Hubl's to be the best method. He deprecates the use of Wijs' method as giving inaccurate results when there is any excess of chlorine present in the solution. He also recommends the Hanus' method for industrial use as it is rapid and gives consistent results. Lewkowitsch on the contrary states that the Wijs' method gives more correct results than Hanus' method which he describes as a 'superfluous addition' to the well tried process of Wijs'. In 1903 Munson and Tolman found that practically identical results



real iodine value is not reached before an hour and a half when it is attained quite suddenly. In case of Wijs' solution the real value is attained within an hour, and the absorption is gradual and within fifteen minutes the value approaches the constant value. Therefore if time is a consideration, the Wijs' is to be preferred to Hanus' especially when other values in addition to iodine value are to be determined with a view to detect adulteration.

*Summary and conclusions*—Wijs' and Hanus' methods of finding iodine value have been studied from three aspects, viz, (1) temperature, (2) concentration, (3) time. Under many conditions both methods give practically the same value, but there are a few instances where Wijs' method gives slightly higher figures. This is due to substitution taking place in the glyceride molecule besides addition. This can, however, be avoided by performing the Wijs' determination at the ice room temperature when its value coincides with that of Hanus' at room temperature. In order to reconcile the values obtained by the two methods both the solutions should be prepared at the 'standard stage,' i.e., when the final thiosulphate titre on passing chlorine or adding bromine is exactly double the titre of the original N/10 iodine solution. An easy method of preparing Wijs' solutions is described. If economy of time is a consideration, Wijs' method is to be preferred to Hanus'. But considering everything, Hanus' method should be chosen on account of the comparative ease with which the solution can be prepared and on account of the fact that there is no chance of its giving higher values in any case owing to substitution as in the case of the Wijs'.

Our thanks are due to Mr Dulal Ch De, BSc, for having made duplicate determination of the values in the Tables III and IV.

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- |                                   |   |
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| HANUS (1901)                      | <i>Zeits f Unters Nahr U Genussm</i> Vol IV, p 913                        |

The higher values obtained in these instances by Wijs' method are due to substitution as well as addition taking place in the glyceride molecules on account of the greater reactivity of iodine monochloride compared with iodine monobromide. The next experiment shows how this substitution can be avoided by altering the experimental condition.

*Effect of temperature on iodine values*—In the literature on the subject, the effect of temperature on iodine values is rather ignored and also its effect on the comparative value of the two methods. Lewkowitsch recommends a higher temperature than 15°C with Wijs' solution, which freezes at this temperature. We, however, experienced no difficulty in working with this solution when kept at 2°C in the ice room. No freezing took place in the mixture of carbon tetrachloride and glyceride. Fryer and Weston (1920) also recommend 20°C as the working temperature of this solution but without assigning any reason.

Apparently both Wijs and Hanus neglected the condition of temperature and the general procedure everywhere is to work at room temperatures which, however, must vary widely. By determining the iodine values at widely differing temperatures, i.e., Calcutta room temperature and 2°C, any effect due to the difference of temperatures is magnified. We took for this purpose the three samples Nos. 10, 9, and 7 (*vide* Table I), whose iodine values from the Wijs' were found to be distinctly higher than those from the Hanus'. In this experiment we made the conditions strictly comparable by adopting the procedure mentioned above (temperature being excepted). The iodine values found from both the methods were distinctly less at the ice room temperature. It is interesting to note that the iodine value from Hanus' solution at room temperature approximates to that from Wijs' solution at ice room temperature. The results are as follows—

Time given 1½ hours in each case

TABLE II

Name of glycerides	Hanus' iodine value		Wijs' iodine value	
	@ 2°C.	@ 33°C	@ 2°C.	@ 33°C.
Linseed oil	177.4	179.1	178.3	180.3
Cocoonut oil	7.8	7.9	8.05	8.4
Mustard oil	94.8	95.8	95.7	96.3

*The effect of concentration of the solutions on the respective iodine values*—The descriptions of methods for the preparation of the Wijs' and Hanus' solutions are somewhat confusing. Lewkowitsch, for instance, states for the Wijs' solution 'A cheaper way of preparing the solution is to dissolve 13 gms of iodine, in a litre of glacial acetic acid, then to determine accurately its content of

the province where climatic, topographical, racial or other conditions might possibly influence the amount of hookworm or other helminthic infections

In most cases a request for assistance was sent to the Directors of Public Health or Chief Medical Officers in the various provinces and states to be investigated, with a copy of the proposed schedule of work. These officers made arrangements with the local health officers or other local officials to give such assistance as was needed, they assisted in the selection of suitable villages in which to work, in obtaining the confidence and co-operation of the village people, and in arranging matters so that a maximum amount of work could be accomplished in a minimum amount of time. In many cases the local officers also actively assisted in carrying out the work, and did a great deal for the personal comfort of the field workers. It is a pleasure to take this opportunity to express my appreciation of the very earnest and efficient assistance which has been given by the health and medical officers wherever and whenever their help was solicited. The deep interest and active co-operation shown by these officers in all parts of the country is a high tribute to the personnel and esprit de corps of these services in India.

The field men went out on tour involving visits to each of the selected localities and picked out one or often two villages in each locality for special study. Assisted by local officials the men made arrangements with the villagers, usually through the medium of the head man or some other influential person, for the collection of stool samples. This was usually done by distributing slips of paper with the age, sex and caste of the individuals marked on them, and sometimes the name also, to be posted beside the stool when passed on the following morning. Samples were then collected in half ounce tins by a sweeper under the direct supervision of the field men, special care being taken to see that the tins were well filled and tightly closed, so that very little development of the eggs could occur even in a period of several days. In this way it was possible as a rule to get identified samples of from 75 to 100 people. In some cases, due to ignorance, prejudice and suspicion the people were unwilling to co-operate, and it was then necessary to collect the samples at random from fresh stools in the defaecation areas around the village, being careful to get approximately correct proportion of samples from men, women and children in case there was any difference in their defaecation areas. The only advantage of the identified over the unidentified samples was the possibility of determining the relative degrees of infection in different ages and sexes. Since in India there is seldom any marked difference between the sexes except in a few places where their defaecation habits differ, and the average infections everywhere seem to advance fairly steadily with age, the disadvantages inherent in the collection of unidentified samples were slight. There was, of course, the possibility of occasionally picking up a sample of a non-human stool, but we are certain that this could very rarely happen, and the men were instructed not to collect samples from stools about the origin of which they had any doubts. This method of collection does, however, leave room for doubt in case of the finding of rare or hitherto unrecorded parasites. For example, we found two stool samples with gnathostome eggs, although adult gnathostomes have not previously been recorded from man. In both cases the stools contained

Strength of thiosulphate solution = 1.02 N/10

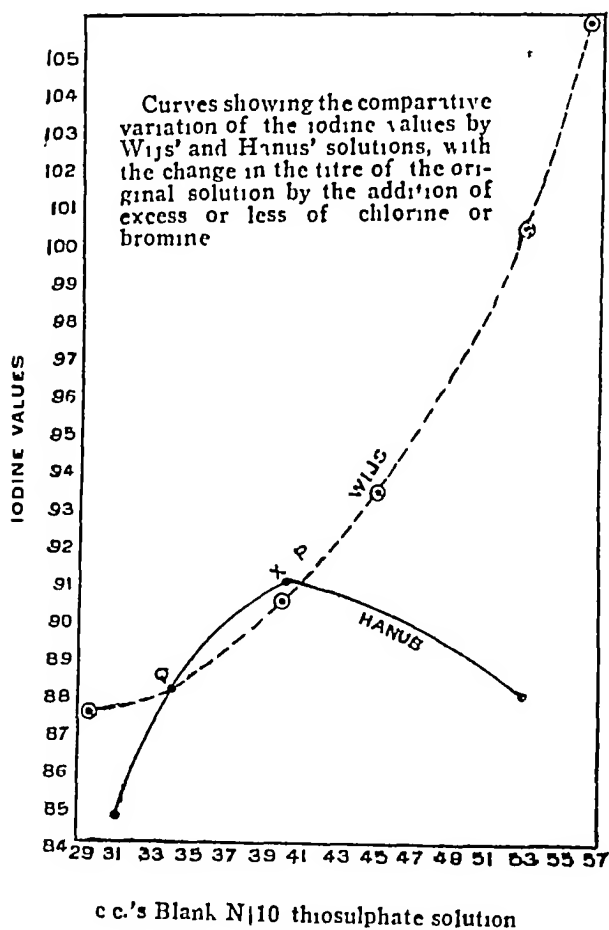


Fig. 1,

The results are interesting and show how the excess or otherwise (over double decinormal) of chlorine or bromine affects the real iodine value. With Hanus' the highest iodine value is reached at the stage when the thiosulphate titre is just doubled. In Wijs' on the other hand the iodine value increases regularly with the increase of the thiosulphate titre of the final solution. But the iodine value obtained from the particular Wijs' solution where its titre is just doubled agrees very nearly with that obtained from the Hanus' solution of corresponding strength. This fact is well illustrated in the graphs. The two curves intersect each other at two points *vide* P and Q. Point P is common to both the curves where both the iodine values and blanks are equal. This point practically coincides with point X where the highest iodine value is reached in case of the Hanus' solution whose thiosulphate titre is just double its original value. The slight deviation of P from X is probably due to experimental error. This clears up the difficulty and doubts in the preparation of Wijs' and Hanus' solutions. In order to reconcile the values obtained from the two methods it is necessary that the thiosulphate titre of the final solutions should be double the titre of the original solution in both instances. For convenience this stage may be called 'the standard stage'. The slight excess of iodine stage as recommended only

his colleagues in Porto Rico and China all egg counts are reduced to the basis of formed stools. In India, however, mushy stools, except in a few hill areas, are the rule, at least 90 per cent of the stools in most parts of India are mushy. Since this type of stool appears to be normal in India, we have not reduced our counts to the basis of formed stools, but have retained them as they were found except in the very few localities where formed stools predominated, and in these places we corrected the counts to the basis of the mushy stools in order to make them comparable with other parts of India. Our counts serve as a direct basis of comparison with counts made anywhere in the plains of India, and also with counts made in Siam and probably Malaya and the East Indian Islands, but they have to be multiplied by two to make them comparable with counts made on the basis of formed stools in America, the West Indies and China.

### GEOGRAPHICAL CONSIDERATIONS

India is a huge country extending from China, Siam and Indo-China on the east to Afghanistan and Persia on the west, and from the snow-capped Himalayan on the north to 8° north of the equator on the south. It contains a population of over 300 millions of people, in the north-west they are predominantly Aryan, in the central and southern parts they are Dravidian, while in the northern hills, Assam, and Burma there is a distinctly Mongoloid strains. Mohammedans predominate in the north-west and in Eastern Bengal, Buddhists in the eastern Himalayas and Burma, Sikhs in the northern Punjab, and Hindus in nearly all other parts of the country. Many of the primitive hill and forest tribes all over India are animists, with no well-defined religion or creed. In spite of this great diversity of racial origin and religion, the habits of the people so far as they affect hookworm disease vary remarkably little, and race, caste and religion can be regarded as playing a very minor part in the epidemiology of the infection.

Topographically every type of country can be found. The lofty peaks of the Himalayas extend all the way across on the northern side. At the foot of these mountains is a great plain, the headwaters of the Indus in the west, the valley of the Ganges in the centre, and the valley of the Brahmaputra in the east. This great plain extends southward to include practically the whole of Bengal which is a vast deltaic plain of the combined Ganges and Brahmaputra rivers. On the eastern side of Bengal and the southern side of the Assam valley are ranges of lesser hills, seldom rising to an elevation of over 6,000 feet, these hills separate Burma from the rest of India, and are inhabited for the most part by wild uncivilized tribes. On the western side the northern plain is extended southward through the Punjab, Sind and western Rajputana and northern Bombay, most of it being in the valley of the Indus. This plain is bounded on the west by the bare rugged mountains of Afghanistan and Baluchistan, and on the east by the outlying spurs of the great central Indian plateau or 'Deccan'. The peninsula proper has a narrow coastal strip on each side, bounded on the western side by a nearly unbroken mountain range, the Western Ghats extending from north of Bombay almost to the tip of the peninsula, and rising to a height of about 3,000 feet, and on the east by a more irregular and broken range, of less

amount of time necessary for the attainment of the real iodine value in both methods

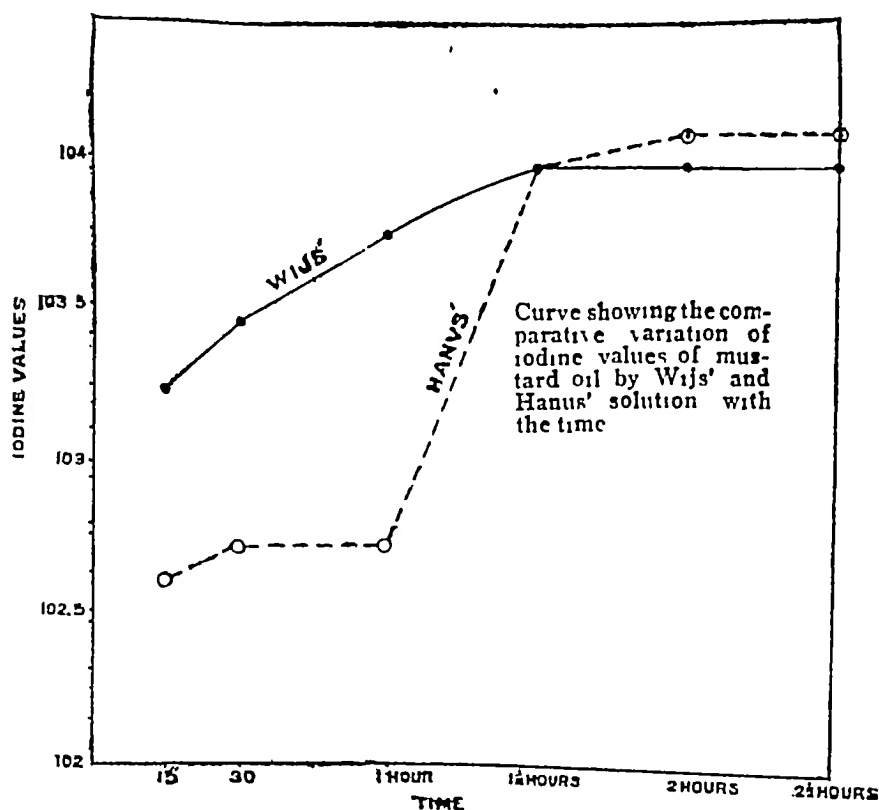


Fig. 2.

The examinations were carried out under exactly the same conditions

After a given interval of time taken by a stop watch, the reaction was finished simultaneously by adding a definite amount of pot iodide solution and water. The results are given below and shown in the graphs in Fig. 2

TABLE IV

Time interval	Hanus' iodine value	Wijs' iodine value	REMARKS
15 mins.	102.6	103.3	Real iodine value attained in case of Wijs'
30 "	102.7	103.5	
1 hour	102.7	103.8	
1 1/2 hours	104.0	104.0	
2 "	104.1	104.0	Real iodine value attained in case of Hanus'
2 1/2 "	104.1	104.0	

It is seen that the absorption is much quicker in case of the Wijs' solution. With Hanus' the values are practically stationary up to one hour's time and the

shortly after the south-west monsoon ceases, and brings rain principally in the months of October, November and December, but it never carries such large amounts of water as the other monsoons

In northern India the year is divided into three very distinct seasons, the cold or winter season, which is practically rainless, extends from about the middle of October to about the first of March, but only the months of December and January are really cold. Frost is very rare except in Assam and northern Burma, and freezing temperatures are unknown except in the Himalaya Mountains at 6,000 feet or more elevation. In the plains mean minimum temperatures in January and February vary from about 40°F to about 65°F and the mean maximum ones from about 75°F to 90°F. While this is a sufficiently low temperature materially to retard the development of hookworm larvæ, it is not sufficient, under otherwise favourable conditions, to stop it entirely. Since, however, the cold weather season is almost rainless except for occasional showers, and the ground never remains moist long enough for the larvæ, with their retarded development, to reach the infective stage, the amount of hookworm infection acquired in this season is negligible. Following the cold weather comes a hot dry season extending in most places from March to about the middle of June. The mean maximum temperatures in this season are in many localities too high to be favourable, since they frequently range from 95°F to 105°F, with the mean minimums somewhere around 80°F. In most parts of northern India there is very little rain in these months, but Assam is exceptional in this respect. The combined effect of the high temperatures and very light rainfall makes the acquisition of hookworm infections practically impossible in most parts of India in this season also. The monsoon breaks about the middle of June in most places, and continues until September. During this season the temperatures in most places which are favoured by the monsoons are moderate, the mean maximum falling between 88°F and 92°F, and the mean minimum about 10°F lower, this comes very near being ideal from the standpoint of hookworm propagation. As long as the monsoons deliver a sufficient amount of rain to keep the ground continuously moist, climatic conditions are highly favourable for hookworm propagation, and in poorly drained areas with high humidity the favourable conditions may last for two months or more after the cessation of the rains.

In southern India there is no true cold season, and the rains are in most places extended over a longer season, with lighter monthly falls. On some parts of the west coast the rains begin in May and last until December, with a partial break in August or September. On the east coast there are light falls during the summer months, and heavy ones in October and November, and sometimes in December also.

Our investigations show that both the total annual amount of rain and the distribution through the year have an important bearing on the propagation of hookworm infections. When the total rainfall falls below 40 inches a year, there may be a high incidence of light infections, but heavy or even moderate infections are absent or very rare, and consequently there is little or no hookworm disease. If the total rainfall is so distributed that not more than 5 or 6 inches fall in any one month the incidence is small also, whereas in places where two, or even

# THE PREVALENCE AND EPIDEMIOLOGY OF HOOKWORM AND OTHER HELMINTHIC INFECTIONS IN INDIA

## Part XII.

### GENERAL SUMMARY AND CONCLUSIONS

BY

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IN view of the fact that very little accurate information has hitherto been available with respect to either the amount or the epidemiology of hookworm and other helminthic infections in India, and that very varying opinions existed as to the necessity for anti-hookworm campaigns conducted on a large scale, an attempt has been made to survey the whole of India in such a manner as to indicate to what extent helminthic infections constitute an important problem in India, which parts of the country are seriously affected, what factors influence the degree of the infections in different parts of the country and what are the most promising ways of reducing and controlling the infections.

The investigation has been made under the joint auspices of the Calcutta School of Tropical Medicine and the Indian Research Fund Association. The regular staff of the Hookworm Research Laboratory of the School of Tropical Medicine carried on all of the laboratory work, whereas the field work was carried out by two especially trained field men whose salaries, as well as all the travelling expenses and other items connected with the field work, were paid by the Indian Research Fund Association.

### METHODS OF INVESTIGATION

The methods which we planned to use were described in Part I of this series, but as the work progressed our methods changed somewhat, so a brief account of them is necessary.

In each province in India a number of localities were selected which were so far as we could ascertain in advance, representative of the various parts of



cease about the same length of time after the ground has become dry. This may be anywhere from two weeks to two months or more after the rains cease, depending on drainage, humidity, soil, and the amount of rain which actually fell. When comparing the degrees of infection in two different places, therefore, it is necessary either that the investigations be made at the same time of year relative to the favourable and unfavourable seasons, or that an adjustment should be made to neutralize differences due to the seasonal factor. When our investigations were made within a month or six weeks of the season of theoretical maximum infection no adjustments were considered necessary, but if made at other seasons we have attempted to estimate the probable reduction in worms harboured and to calculate an index of infection based on the season of maximum infection. In our bar diagrams showing the relative amounts of infection, corrections have been made to neutralize this seasonal factor.

### OCCUPATION IN RELATION TO DEFÆCATION HABITS AND HOOKWORM INFECTION

Occupation does not play such an important part in the epidemiology of hookworm in India as the Hookworm Commission in China has shown to be the case there. About 90 per cent of the entire population of India is classified as rural, and the vast majority of these follow agricultural pursuits. In China, hookworm infection is influenced to a very minor extent by ordinary soil pollution (Cort, Grant and Stoll, 1926) and is dependent for the most part on the subsequent utilization of human faeces as fertilizer. In consequence, the amount of hookworm infection depends very largely on the extent to which the people come in contact with ground fertilized in this manner. The infection in China is not only rural but agricultural and even varies to a very large extent with the type of crop and the methods used in producing it. No such condition exists in India. Night-soil is practically never utilised as fertilizer except incidentally as the result of soil pollution. Soil pollution, on the other hand, is an almost universal habit except in a few special localities and is the habit of nearly all the rural people without respect to race, religion, caste or occupation, in most places only a very few of the better educated high-caste people have adopted the use of latrines. These are so few in number that they can be ignored for all practical purposes. In the delta division of Burma, however, in localities which are largely flooded during the monsoon season, latrines are almost universally used by all but the Indian settlers. In many cases these latrines are very primitive, in some places they are merely shallow pits, with a few logs laid across the top, while in other places there is a superstructure built so that the squatting places are several feet above the surface of the ground. In some parts of Eastern Bengal primitive latrines are extensively used by the women and children. These are even more primitive than in Burma, and usually consist merely of a sloping bamboo pole, with or without a screen of dead leaves, or of a convenient branch or raised root of a tree. There is seldom any attempt at a pit. In some of the Mohammedan villages of the northern United Provinces and the Punjab the women use special mud closets for defæcation. There may

other eggs characteristic of human stools and had every appearance of being human stools. We feel reasonably confident that these two cases represented true cases of human parasitism with adult gnathostomes yet we are unable to record it as an absolutely certain fact.

On the date of collection the samples were posted to Calcutta, and placed on ice upon their arrival there. Usually it was possible to examine them immediately, and we are sure, from the appearance of the eggs, that in very few cases was any appreciable number lost through hatching or deterioration.

Meanwhile the field men made as careful an epidemiological study of the villages as possible, both by personal observation and by questioning of the people themselves. A sketch map of the village was drawn showing arrangement of houses, defaecation areas, water courses, tanks, wells, etc. Copious notes were taken on the location and nature of the defaecation areas, the defaecation habits of the people and differences in habits between the sexes. The water supply was carefully studied with special reference to the possibilities of gross pollution and the consequent acquisition of *Ascaris* and *Trichuris* infections. Notes were taken on the arrangement and construction of the houses from the point of view of rats and the acquisition of *Hymenolepis* infections, and the food habits were also studied from this standpoint especially the extent to which cooked food was kept overnight, and the care taken to prevent access of rats to it. Notes were also taken on the kinds, number and habits of domestic animals and on any other factors which might influence helminthic infections. At the beginning of the work in Bengal soil infestation studies were made by the collection of samples from defaecation areas and from other spots which were suspected of being possibly infested, and fresh stool sites were marked for the collection of soil samples a week later, the number of eggs per gram in the stools so marked being determined in the meantime. It soon became evident that little further information could be obtained by continuing this work, and that it was not worth the time required for it, since our studies in a few localities made it possible to say with a fair degree of accuracy from observation alone the amount of infestation which would develop in any given place.

After the men had completed their work in the selected localities on each tour, I made a personal visit to the majority of them to check upon their work, and to observe the conditions at first hand. With the aid of the notes, it was usually possible to make this inspection and get a very good picture of epidemiological conditions in the space of a few hours. Without the soil infestation studies, it was possible for the field men to get their stool sample and to collect all the necessary epidemiological data in from two to three days.

In the laboratory the stool samples received were examined for incidence of infection by the direct centrifugal flotation method (Lane, 1924) and positive then examined by a modification of Stoll's dilution egg count method (Chandler, 1925) to determine the degree of hookworm infection. In case 90 per cent or more of the samples were positive in the first lots examined by D C F, egg counts were made on all the samples first, and only the negatives examined by D C F. Stoll (1924) has shown that on the average formed stools contain twice as many eggs per gram as mushy stools and in his work and in that of

almost exclusively confined to rural communities or people living under rural conditions

There is no evidence that occupation, *per se*, has any appreciable effect on the degree of hookworm infection except in a very few special cases, e.g., tea garden labourers, miners, sewage farm coolies, etc. In some cases the occupation and the amount of hookworm infection is jointly affected by other factors, e.g., sea fishermen have comparatively little hookworm, because they live in proximity to the shore, and cotton producers because the soil which is favourable for cotton is unfavourable for hookworm propagation. In a given village we have found no significant difference in the amount of hookworm in farm labourers, shop-keepers, cobblers, or people of other occupations. They all use the same defæcation areas, and are all about equally exposed to infection.

Tea garden coolies appear to be rather more severely affected than do the people in neighbouring villages who do not work on the gardens. This appears to be due in part to the more favourable condition of the soil for the development of hookworm larvæ as the result of frequent cultivation, and in part to the more frequent contact with polluted areas during the plucking season, which corresponds with the season favourable for hookworm propagation. On most tea gardens in India every bush is plucked once every two weeks, and consequently contact with any polluted soil in the gardens is ensured within the time of survival of the larvæ. Tea garden coolies dislike plucking in the vicinity of coolie lines on account of the ground itch which they recognize to be a consequence of plucking in such localities.

Miners are also exposed to an unusual degree since the warmth and humidity in the mines does away with the desiccation which occurs on the surface throughout the dry season of the year. If it were not for the devouring of stools by roaches and the consequent destruction of a high proportion of the eggs contained in them (*see* Chandler, 1926), the acquisition of infection in underground defæcation areas would be much greater than it actually is.

Sewage farm coolies are exposed practically throughout their working hours to soil in which there is a widespread, though usually fairly light, infestation with hookworm larvæ. Even in parts of India where practically no infection results from ordinary surface pollution (e.g., Sind), the sewage farm coolies have a high degree of infection. There appears to be no remedy for this except an annual mass treatment of such coolies.

## OTHER EPIDEMIOLOGICAL FACTORS

### FOOTGEAR

The wearing of shoes undoubtedly has an important influence on the amount of hookworm infection (*see* Davis, 1923), but it happens that in most parts of India where climatic conditions are favourable for hookworm, shoes are rarely worn except on special occasions or on visits to other villages or towns. In some parts of the United Provinces and Central India the wearing of footgear, usually in the form of *kharams*, wooden soles without uppers, especially by the men, has a beneficial effect. In many parts of north-west India, also, footgear is

elevation, the Eastern Ghats. Between these ranges is a great plateau with an average elevation of from 1 500 to 2,500 feet, known as the Deccan. On the north, between the Deccan and the plains of the Punjab and United Provinces, the country is irregularly broken by hills, plateaus and valleys, while in the south there is also a rather irregular arrangement of groups of hills and intervening plains. In Burma there is a deltaic plain of the Irrawaddy, and a number of more or less parallel valleys running north and south, separated by hills of moderate elevation, for the most part not over 3,000 feet. The Shan States and the northern and eastern parts of Burma are all hilly, with few plains of any appreciable extent.

### CLIMATIC CONDITIONS IN RELATION TO HOOKWORM INFECTIONS

The climatic conditions vary enormously in different parts of the country, and it is this variation which is the keynote to the very uneven distribution of hookworm infections. Since the climate has been discussed at some length in the various sections dealing with different geographical divisions of the country, it is only necessary here to give a brief review of the climate and the factors influencing it, in the country as a whole.

Practically the whole of India except the mountains at an elevation of about 5,000 feet or more in the north has a tropical climate. Throughout India the rainfall is seasonal, the rain being carried by several distinct monsoon currents. The south-east monsoon sweeps up across the Bay of Bengal and supplies practically the entire rainfall for Burma, Assam, Bengal, Bihar and Orissa, eastern Central Provinces, United Provinces, and the Punjab. It breaks in Burma and Assam in May, and somewhere between the beginning and end of June in other places. The precipitation is very heavy on the coasts and near the foot of the mountains, and gradually decreases toward the north-west. This monsoon gradually dies in September, though in some localities there may be some precipitation in October also.

The western side of India is supplied with rain by the south-west monsoon, which sweeps in a north-east direction across the Arabian Sea, and strikes the west coast of India. The heaviest precipitation is in the south, along the Malabar Coast, but there is heavy rain on the coast as far north as the Western Ghats, extend. These mountains cause the monsoon winds to drop most of their moisture, so that the Deccan just east of the crest of the mountains is very dry. Where the Western Ghats cease in northern Bombay the coastal rainfall is greatly reduced and a fair amount of rain is carried inland to central India. In parts of Central India and the Central Provinces there is a sort of no-man's-land between the two monsoons to which one or both or neither may reach in any particular year, consequently the annual rainfall is subject to relatively enormous fluctuations. A moderate amount of rain crosses the Western Ghats in the south so the central part of southern India is not so dry as the Deccan. The south-west monsoon breaks in May and extends to August.

The north-east monsoon sweeps southward over the southern half of Madras, affecting principally the Coromandel Coast and Travancore. It becomes active

direct experiment (Chandler, 1926c) that only a small proportion of the eggs eaten succeeded in passing through the grinding organs of the proventriculus of these insects. There can be no doubt but that roaches are highly beneficial in controlling hookworm infection in mines which have no sanitary arrangements.

Dung beetles (Scarabæidæ) constitute a very important factor in the development of hookworm larvæ. Through the entire season during which there is moisture in the soil, and therefore the season when larvæ can develop, dung beetles are very active in stirring up stools with soil, the result being that excellent cultural conditions are produced, and the larvæ develop far more readily than if the stools were left untouched. There is no doubt but that the stirring up of stools by dung beetles very greatly favours the development of hookworm larvæ from eggs contained in them. As a rule in moist soil it is only a matter of a few hours, almost never more than a day, until the site of a stool has the appearance of a mound of finely granulated earth due to the activity of these insects, the surface of such mounds always contains more hookworm larvæ than would be found on the site of undisturbed stools on the surface of the ground.

#### SOIL.

The nature of the soil unquestionably has an important influence on the degree of infection. Experiments recently conducted by the International Health Board in Alabama (1924) have shown a striking correlation between the amount of hookworm present and the type of soil. In the Report for 1924 the following statements are made: 'The hookworm distribution follows the type of soil very closely, and there is little overlapping. For example, at the dividing line between the sandy coastal plain and the black soil belt, one community in the sandy belt was found with every child infected, most of them heavily, whereas in a similar community in the black soil belt, only two or three miles away, not a single child was infected. The type of soil is therefore of great importance in determining the intensity of hookworm infection in a community. An experienced observer should be able by a glance at the soil of the area, providing he knows the mean temperature and rainfall of the region, to determine whether there is, or ever can be, a real hookworm problem there.'

Heavy clay soil has long been recognized as an unsuitable medium for the development and survival of hookworm larvæ. Patches of such soil occur in nearly all parts of India, but they are usually not very extensive. Extending across the north central portion of Bengal there is a fairly broad area of red clay known as the 'barind,' and a red clay which attains a slippery surface when wet occurs extensively, in the Assam hills. Black cotton soil is particularly unfavourable for hookworm larvæ, it requires over twice as much water to make it wet as is required for ordinary alluvial soil, it consists of very fine clay particles through which hookworm larvæ cannot readily migrate, and it tends to dry into extremely hard clay-like clods when exposed to the sun and dry atmosphere of the parts of the country where it is found. I do not believe any area with this type of soil will be found to have any appreciable amount of hookworm infection. Black cotton soil occurs very extensively in the Central

only one month, has nine or ten inches or more the incidence may reach 70 per cent to 80 per cent. The monthly rainfall which is necessary to allow hookworm propagation depends on a number of other conditions, the most important of which are drainage, type of soil, humidity, and number of rainy days over which the rainfall for the month is distributed. If the drainage is poor, the soil not too light, the humidity high, and 9 or 10 or more days in the month are rainy (i.e., with at least 0.1 inch of rain), five to six inches appears to be quite sufficient, whereas under less favourable conditions at least seven inches appears to be required. The larger the number of months in the year in which the ground is kept practically continuously moist, other things being equal, the greater the amount of hookworm which will occur. We have adduced evidence (Chandler, 1926) to show that hookworm infections are rapidly lost when re-infection is stopped. Three months of dry weather appears to be sufficient to ensure a loss of about 50 per cent of the worms harboured, 60 per cent are lost in six months, and 65 per cent in 9 months. The consequence of this is that a dry season interferes with the cumulative acquisition of worms, the time during which hookworms can be acquired is reduced in most parts of India from 12 months to from 2 to 8 months, a fairly high percentage of worms is lost in the interim between favourable seasons, and at the beginning of each succeeding favourable season the number of eggs reaching the soil, and therefore the intensity of soil infestation, is correspondingly reduced. A dry season of from 6 to 8 months during which hookworm re-infection is practically entirely stopped can be regarded as having very much the same effect as a mass treatment, in fact the degree and distribution of hookworm infections in most communities in India are strikingly like those which are found in countries with more favourable climates after an extensive treatment campaign, i.e., there is a high incidence of light infections with very few if any heavy infections. In Porto Rico, for instance, after a thorough treatment campaign in a severely infected district, Payne, Cort and Riley (1923) found the infections distributed as follows: Negative to egg count, 33 per cent, 50 to 250 eggs per gram (reduced to the basis of mushy stools to be comparable with our Indian counts), 37 per cent, 300 to 1,000 eggs per gram, 25.7 per cent, 2,500 to 5,500 eggs per gram, 2.9 per cent, and 5,500, 1.4 per cent. Such distribution of infections is typical of the greater part of India where hookworm infections are prevalent, e.g., throughout the plains of Bengal, Bihar and Orissa, eastern Central Provinces, and United Provinces. Cort et al (1926) call attention to a similar resemblance of the hookworm infections in some parts of China, where the climate is unfavourable, to the amount of infection left in heavily infected places after a successful treatment campaign. Nowhere in India are there infections as heavy as those found, for instance, in the West Indies or even in favourable localities in south-eastern United States, the heaviest infections occur in a small part of Burma, the Assam valleys, the Darjeeling district of Bengal, and on the southern Malabar Coast—all places where there are seven to eight favourable months in the year.

Since it requires about six weeks after infection for hookworms to begin depositing eggs, the egg counts begin to show the effect of the new acquisition of worms about this length of time after the beginning of the rainy season, and

places, however, there are differences. It frequently happens that the females use localized areas close to the villages, where groves or thickets afford protection from view, while the men have less well defined and more scattered defæcation areas at greater distances. The places frequented by the females consequently become more heavily polluted and a higher degree of infection in this sex results. In other places the females habitually use latrines, while the males go to widely scattered places in the fields and jungle, in this case the degree of infection in the females is less than in the males. On the tea gardens the females are usually slightly more heavily infected than the men, probably due to additional exposure to infection while plucking the bushes. Our own observation, as well as Dr Kendrick's very large series of egg counts in Madras Presidency, show conclusively that there is a fairly steady average increase in the degree of infection with advancing age. Children become infected very soon after they begin to run about, but since they are more promiscuous in their habits and do not so regularly visit the defæcation places used by the adults, they are exposed to infection less frequently than the latter. The habits become more and more fixed with advancing age, and we believe that the increasing regularity in the visits to definite places for defæcation is quite sufficient to account for the steady increase in degree of infection with advancing age. Smillie's (1922) suggestion that it is due to a slow acquisition of infection we believe to be entirely untenable, for we are convinced that infections are rapidly acquired and rapidly lost.

#### SPECIES OF HOOKWORMS

The species of hookworms which affect man in India differ somewhat in different parts. In southern India *Necator americanus* is practically the only species present, less than 5 per cent of the total number of worms harboured are *Ancylostoma duodenale*, and *Ancylostoma braziliense* has not yet been recorded in man in that part of India. In Bengal, Chota Nagpur, Orissa, Assam and the central parts of India *Necator americanus* is still the predominant species, but the proportion of *Ancylostoma duodenale* is higher. In Bengal, roughly about 20 per cent of the worms harboured are of this species. According to Korke (1926) *A. duodenale* becomes still more common in Bihar, where it actually becomes the predominant species. Unfortunately our methods of investigation do not demonstrate the species involved, but we consider it very probable that a relatively high proportion of the worms harboured in the United Provinces and in the Punjab, as well as in Bihar, are ancylostomes rather than necators, our reasons for believing this are that the number of eggs per gram and the clinical severity of the infections in these places are greater than would be expected in comparison with Bengal, in view of the less favourable climatic conditions. With a higher proportion of ancylostomes, a smaller number of worms would be required to produce these conditions than would be the case in Bengal, where about 80 per cent of the worms are necators. If we are right in this surmise, it would appear that *A. duodenale* is much more prevalent among peoples of Aryan origin, or with a considerable admixture of Aryan blood, than it is among the Dravidian and Mongolian races. Jolly's investigations in Burma

or may not be a log or board to stand on with a shallow ditch behind, in many cases the stools are passed merely on the surface of the ground, but as these stools are usually removed daily the danger of infection is not very great. In some parts of Bengal it is a common practice for the men, during the flooded season, to defæcate over the sides of the primitive boats which they use in crossing their paddy fields. In many parts of southern India there is a piece of ground set apart inside the village, and surrounded by low walls, as a defæcation place for women. Although called a latrine by the local people, such a place has none of the advantages of a latrine, and its use must be more dangerous than promiscuous soil pollution.

With these few exceptions, the village people of India habitually defæcate on the surface of the ground somewhere near the villages. Usually the nature of the environment is such that certain general localities where there is protection from view, and often where water for ablution is handy, become more or less well-defined defæcation areas. Localities which are especially favoured are groves of bamboo, mango, prickly pear, or other shrubs or trees near the village, the sloping banks of tanks, streams or ditches, the dry bed of streams, corners of cultivated field, the sides of roads or paths. In some places the men and women use the same location, but in many places the men go to somewhat more distant places than the women, and their defæcation areas are often, in consequence, more scattered and less well defined. In compact villages such as are found in the United Provinces and central and north-west India there are general defæcation areas where people from a whole section of the village, or even the entire village, mingle. In parts of Eastern Bengal, Assam, northern Burma, the west coast of southern India, and in the Himalayas, where the villages are scattered, with considerable intervals between houses or groups of houses, there are often separate defæcation areas for each household, or at least for each small group of households.

It is obvious that the more concentrated the defæcation areas, the greater the danger of contact with infested ground when the areas are visited. We are convinced from our epidemiological studies that the vast majority of worms are acquired while standing on infested ground during the act of defæcation. Even the use of a raised root of a tree, or of a low horizontal branch, in so far as it is efficient in keeping the feet off polluted ground during the act of defæcation is a powerful factor in controlling hookworm infection. When there are individual defæcation areas for each household there is likely to be a very wide range in the degree of infection in these different households, whereas in localities where all the villages mingle in a common area the degree of infection is likely to be much more nearly uniform.

In many of the towns, especially in southern India, sanitary conditions are little better than in the villages, but they are rapidly improving, and a bucket latrine system with a subsequent burial in a trenching ground is the commonest method of conservancy met with. In northern India hookworm infection is of no consequence in any of the larger towns or cities, and is much less important in south India than it was a few years ago. Our present studies in the



constantly to make good the damage done by the worms, and as a result it is readily conceivable that resistance to fatigue, exhaustion and disease may be proportionately lowered. Only when the point is reached where the repair cannot keep pace with the damage will any obvious or measurable symptoms be present. Once this point is reached, any increase in the degree of infection will produce an accelerating increase in the damage to the host.

The degree of infection required to produce measurable injury to the host depends, as one would expect, on many other factors. The most important of these is nourishment. Smillie (1922) gave some very striking figures showing the influence of good nourishment on the effect produced by hookworm infection. On a fazenda in Brazil he compared two groups of farm labourers who were living under practically identical conditions except that the individuals in one group, who were milkers, drank several litres of milk a day in addition to their other inadequate diet. The milkers, with an average of 350 hookworms each, had a hæmoglobin index of 66 per cent, which is normal for Brazilian labourers, whereas the field workers who did not have the milk, with an average of only 224 hookworms each, had only 57 per cent hæmoglobin. It has been the experience of practically all observers that it is the undernourished individuals who suffer most from hookworm disease. Overwork, excesses, hardships of any kind, and chronic diseases, all tend to increase the effects of hookworm infections, just as hookworm infections tend to render the individual more susceptible to these other factors. They all require vitality to resist them, and if several of them occur together, the odds against the patient are much greater. Age also is an important factor. The powers of compensation are not as great in children or in people past the prime of life as they are in young or middle-aged adults. There is some evidence that race also has some influence. It has been the experience of myself and others that negroes in southern United States suffer less from equal degrees of hookworm infection than do whites, and Gordon's (1925) recent investigation in West Africa gives further evidence of the relative immunity of the negro race. We have no definite basis for a statement of the relative susceptibility of Indians or of different races of Indians, but casual observation of very large numbers of individuals has led me to the opinion that the Dravidian races are somewhat less susceptible than either the Aryan or Mongoloid races, but more so than the negroes.

It is not surprising, when all these factors are taken into consideration, that there should be no close correlation between the degree of infection and the severity of infection in individuals, although a certain amount of correlation can be observed in communities. In Brazil, Smillie (1922) found a definite break in resistance, with a measurable loss of hæmoglobin, when the average number of worms reached about 75. In southern United States, Smillie and Augustine (1926) found no effect produced in school children by infections of from 1 to 25 worms, exceeding little by infections of 26 to 100 worms, but observed definite improvement after treatment in children with 100 to 500 worms, and very marked improvement in children with over 500 worms. They arbitrarily divide the infected individuals into 6 groups as follows: 1, no hookworms, 2, very light infestations, 1 to 25 worms, 3, light infestations, 26 to 100 worms, 4, moderate

extensively worn both by men and women, but there the climatic conditions are such that no appreciable degree of hookworm infection could develop even if shoes were not worn. The low degree of infection in the Shan States is almost certainly due to a large extent to the habitual wearing of shoes. In other parts of Burma, as well as in Assam, Bengal, Bihar and Orissa, and in southern India shoes are rarely worn.

#### DOMESTIC ANIMALS

The effect of the devouring of human faeces by domestic animals is a question about which there is a considerable difference of opinion. Ackert and Payne (1922) were the first to demonstrate the fact that when human stools are eaten by pigs, the eggs pass through unharmed and subsequently develop into normal larvæ. Chandler (1924) confirmed this in India, and also showed that worm eggs devoured by dogs and rats were uninjured by passage through the digestive tracts of these animals. Ackert (1922) showed, however, that a high proportion of eggs devoured by chickens were destroyed, presumably by grinding in the gizzard. Where the devouring of stools by pigs and dogs is beneficial or otherwise, therefore, resolves itself into a question of whether the droppings of the animals fall in places where the larvæ which subsequently develop are more, or less, likely to cause infection. Our observations indicate that this depends entirely on local conditions. In some places the pigs and dogs range over a wide area, and the eggs are more widely scattered than if they had not been interfered with, while in other places the animals stay fairly close to the houses or villages, and for the most part drop their stools in much the same places as do the villagers. In this case the devouring of the stools can have very little effect, beneficial or otherwise. It is only when the stools are eaten from latrines or standing places where the concentration of the faecal matter would prevent any high degree of survival of the larvæ, and where human feet would be unlikely to come in contact with them anyway, and are then dropped in places where the developing larvæ are likely to be stepped on, that any damage can be done. For the most part our observations indicate that the devouring of stools by pigs and dogs has very little effect of any kind on the amount of infection. Cattle and buffaloes also avidly devour human stools, but in this case there is room for serious doubt as to whether the eggs survive. So far as I am aware no direct experiments have been made to determine the effect on hookworm eggs of passage through these animals. We have observed, however, that in places where the stools are habitually eaten by cattle and buffaloes the degree of infection is lower than would be expected in consideration of other circumstances, furthermore, we have examined by the D C F method a considerable number of cattle and buffalo stools found on very frequently used defæcation areas where very numerous stools were daily eaten by the animals and have failed to find any hookworm eggs, nor have we succeeded in obtaining any hookworm larvæ from cultures made from these cattle stools.

#### INSECTS

In coal mines we observed that the stools deposited underground were for the most part completely devoured by cockroaches, and we demonstrated...

50 worms Smillie (1922) in Brazil concluded that on the average 75 worms might be considered the degree of infection necessary to produce measurable symptoms, and Smillie and Augustine (1926) in Alabama found extremely little effect in their group harbouring between 25 and 100 worms. Since Indians appear to be at least as resistant as whites, less than 50 worms may on the basis of this work be considered practically harmless. Our own observations as well as those of others in India strongly support this view. Our group 2 with less than 100 eggs per gram can be considered as of very little practical importance even from an epidemiological standpoint, since we have found that the number of larvæ developing on the site of stools containing such a small number of eggs is very small indeed. Our group 3, with from 100 to 500 eggs per gram, while clinically negligible, is of some importance from the standpoint of the spread of the infection. The individuals in group 4, with from 600 to 2,000 eggs per gram, while not entirely negligible from a clinical standpoint, probably suffer very little as a rule unless under-nourished, overworked, or suffering from such chronic diseases as tuberculosis, malaria, kala-azar or leprosy. We consider it very likely, also, that infections of this grade may be injurious in slightly lowering reserve vitality and power of resistance to fatigue and other diseases, especially in the poorly nourished, without necessarily producing any observable symptoms. Group 5, with from 2,000 to 5,000 eggs per gram, corresponding roughly to from 160 to 400 necators, is very similar except that the proportion of individuals showing mild symptoms may be presumed to be greater, and probably most of them suffer from slightly reduced mental and physical endurance and efficiency unless very well nourished and in otherwise excellent condition. Individuals in group 6, with from 5,000 to 10,000 eggs per gram, probably all suffer to some extent, and those in group 7, with over 10,000 eggs per gram, may be considered, with few exceptions, to show distinct and observable clinical symptoms, in many cases severe. To sum up, group 2 is practically entirely negligible from both a clinical and epidemiological standpoint, and may for all practical purposes be classed as negative; group 3 is clinically negligible but of some epidemiological importance, group 4 is on the border line clinically and important epidemiologically, group 5 may be practically unharmed clinically in well nourished and otherwise perfectly healthy individuals, but suffers under any adverse conditions, and only groups 6 and 7 need be considered as having well-marked hookworm disease.

### THE INDEX OF INFECTION

In order to make it possible to make a direct comparison of the actual amount and severity of hookworm infection in different places, we have attempted to work out an index of infection. The incidence of infection alone is of very little value by itself, and we also believe that a statement of the degree of infection alone, based on the average eggs per gram for the community although a far more valuable basis of comparison than the incidence of infection, is also inadequate without a consideration of the distribution of the infections (*see* Chandler, 1925). We thought, therefore, that we could arrive at an arbitrary

Provinces and Central India in parts of the Deccan of Bombay Presidency, Hyderabad and Madras, and on some of the Mysore tablelands

Very light sandy and gravelly or light sandy loam soils are also unfavourable for hookworms in places where the rainfall is small in amount and irregular in distribution. Such soils allow too large a quantity of water to percolate to deep layers, and have too deep a subsoil water level, and they tend to dry out too quickly at the surface. Patches of such soil are found especially in northern Bihar and in many parts of Central India and the Deccan. In Bengal, Bihar, United Provinces and the greater part of the Punjab alluvial soils predominate. These are mixtures of sand and clay in varying proportions, when either element is in strong predominance, conditions are less favourable for hookworm larvæ than when there is a fair proportion of each. Laterite soils are favourable as a rule, they occur in most hilly areas of India.

We are convinced that salt in the earth, resulting from saturation by sea water, has an injurious effect on hookworm larvæ, in spite of the fact that the developed larvæ can survive a long time in brackish water. Our investigations at Gosaba in the Sunderbans point very strongly to this conclusion. Defæcation on a seashore leaves little opportunity for infection, if the stools are deposited near enough the shore so that they are reached by the water at high tide the eggs and larvæ are washed away, if deposited out of reach of the water, the very rapid drying of the sand between showers, the open exposure to the sun, the frequent migration up and down as a result of heat and cold and alternate drying and wetting, the absence of dung beetles to stir up the stools with the sand, as well as the presence of salt in the environment, all tend to prevent the development and shorten the lives of the larvæ.

#### IRRIGATION

Artificial irrigation as a rule has little effect on hookworm infection, for the reason that almost invariably the people prefer dry places on which to stand for defæcation, and even where a relatively large proportion of the land is kept moist by irrigation, there are nearly always dry spots, especially close to the villages, and therefore nearly all the stools are deposited in places which are quite as dry as if there were no irrigation. In Bombay Presidency it is illegal to carry irrigation water within a quarter of a mile of a village on account of danger of malaria, and this almost completely eliminates the possibility of the irrigation water having any effect on the development of hookworm larvæ. Only in a few of the valleys of the north-west frontier is the water brought to the very edge of the villages, and it is only in these places that any effect of irrigation on the amount of hookworm infection could be found. Contrary to the opinion which is sometimes expressed, there appears to be little danger that an extension of irrigation in the dry parts of India will bring about any appreciable degree of hookworm propagation.

#### SEX AND AGE

In most places in India there is no very great difference in the degree of hookworm infection in the two sexes, since their habits are much alike. In some

of all the facts involved in the incidence, severity and distribution of the infections when all the individual cases are studied

Our observations indicate that when an index of infection, worked out as described above, falls below 200, the amount of hookworm present is so small that it is of no practical consequence and can safely be ignored as an important public health problem. When the index falls between 200 and 250, hookworm may be regarded as at least a potential danger to the community, and a small percentage of the people, usually between 2 per cent and 5 per cent, will show obvious clinical symptoms of hookworm disease, usually somewhere between 30 per cent and 50 per cent of the people will harbour a sufficient number of worms so that under otherwise adverse conditions, such as malnutrition, overwork or chronic disease, slight injury may be expected. When the index falls between 250 and 300 the number of individuals who show distinct symptoms of hookworm disease is usually increased to from 5 per cent to 10 per cent and a still higher percentage are on the border line where their infections are likely to be harmful under adverse conditions. In other words, when the index of infection falls between 200 and 300, the hookworm situation is worthy of consideration as a minor public health problem, the importance of which may be regarded as increasing in direct proportion with the index of infection between these points, and in inverse proportion with the hygienic standards of living. When the index of infection falls above 300, we believe that hookworm infection constitutes a really important public health problem which must be attacked before a healthy community can be hoped for. With each increase of 50 in the index of infection above 200 the percentage of individuals who show obvious symptoms of hookworm disease may be roughly estimated to be about 5 per cent and the number of individuals who are on the border line where their infections may be considered injurious to them under adverse conditions may be roughly estimated to increase by about 10 per cent. The urgency of the problem increases in direct proportion with the increase in the index of infection.

#### AMOUNT OF HOOKWORM INFECTION IN INDIA AND ITS SIGNIFICANCE

It is the general opinion that hookworm constitutes a very important problem in India. Incidence statistics, on which our knowledge of the disease in India has been based almost exclusively until recent years, indicated a very prevalent infection and this has led to the assumption that it was also severe. The severe hookworm infections observed in Indian coolies on estates in other countries was thought to indicate a severe degree of infection among them in their own country, and it has even been assumed that hookworm was being imported wholesale by Indian labourers into such countries as Ceylon, Malaya and the East Indian Islands. This, in fact originally led to a wide international interest in hookworm infections in India, resulting in the co-operation of the International Health Board with the Governments of India and Madras in an extensive investigation of the infection in southern India. A summary of our knowledge of hookworm infection in India was compiled by the International Health Board in 1922.

have shown that about 5 to 6 per cent of the worms harboured there are *A. duodenale*, and no less than 38 per cent are *A. braziliense*. This relatively high proportion of this latter species in Burma (and also in Malaya and some of the East Indian Islands) is a very peculiar and interesting fact indicating some special epidemiological factor favouring this worm in these countries and not in other parts of India, what this factor is we are not at present able to say.

### GRADES OF INFECTION AND THEIR SIGNIFICANCE

We have divided our infection into a series of groups according to the number of eggs per gram, as follows: 1, entirely negative, 2, less than 100 eggs per gram, 3, 100 to 500 eggs per gram, 4, 600 to 2,000 eggs per gram, 5, 2,100 to 5,000 eggs per gram, 6, 5,000 to 10,000 eggs per gram, and 7, over 10,000 eggs per gram. As the result of his work in Porto Rico, Stoll (1923) estimated about 25 eggs per gram per female worm in mushy stools and predominant necator infections, and Sweet (1925) estimated a similar number in Ceylon. From estimates which we have made in Calcutta comparing egg counts with worms expelled after treatment, we believe that this number is sufficiently accurate for Bengal also. Roughly, therefore, 100 eggs per gram in our counts can be interpreted as representing 8 necators. Cort et al (1926) in China and Sweet (1924) in Australia have come to the conclusion that *Incyllostoma duodenale* deposits several times as many eggs per day as does *Necator americanus*. Dr Kendrick in Madras informs me that he is of the same opinion and we ourselves have some evidence to the same effect. A hundred eggs per gram, therefore, would represent a much smaller number of ankylostomes. Ankylostomes, however, are at least twice and we believe at least three times as harmful as necators; therefore 100 eggs per gram may be considered for the present as representing, roughly, approximately the same degree of severity of infection whether necators or ankylostomes are present, or any proportion of the two. Furthermore, in children, since the stools are smaller, the concentration of the eggs is greater, therefore 100 eggs per gram would represent fewer worms than in adults. But since there is good evidence that a given degree of infection is more harmful in children than in adults, again a statement of the number of eggs per gram gives a fair rough indication of the severity of the infection as compared with adults. We believe therefore that a statement of eggs per gram gives a much better basis of comparison than does an estimate of the number of worms present and for this reason we have not interpreted our egg counts into terms of number of worms.

The degree of infection which can be considered harmful cannot be fixed except very roughly. There are those who look upon any hookworm infection as definitely pathogenic, but recent investigations have, fortunately, made this opinion practically untenable. No doubt even a single hookworm does some injury to its host, but this injury, in a normal individual, is so easily compensated that it is of little or no consequence. Even when no evidence or measurable symptoms are present, however, we consider it possible that hookworm infections may not be entirely negligible. It takes a certain amount of reserve vitality

Davis' (1924) findings in what he speaks of as a lightly infected area in Brazil. Only one single infection in 287 examinations in villages in the Brahmaputra valley exceeded 5,000 eggs per gram, and only three others fell between 2,000 and 5,000. In villages in the Surma valley the average incidence in group 6 was 3.8 per cent, and in group 5 3.9 per cent. On Assam gardens the degree of infection is practically the same as in the villages, although on a garden studied in Cachar the infection, in spite of the fact that practically no treatments or control measures had been instituted, was surprisingly low in the group studied.

In the hills of Assam the degree of infection is in most places distinctly lower than in the valleys, and in the places investigated varied from an index of 80 to 212. Nevertheless, not a single infection was found among 271 hill people examined in which the eggs per gram exceeded the 600 to 2,000 group. For all practical purposes, therefore, hookworm may be ignored in the Assam hills just as in the Burma hills.

#### BENGAL AND BIHAR AND ORISSA

In Bengal the incidence of infection is high, averaging about 80 per cent, but the degree of infection everywhere in the plains, except in the Dooars tea gardens, is very low, it corresponds with what is found in a heavily infected area after a successful treatment campaign. In the south central portion of Eastern Bengal where latrines or standing places are in general use, the index of infection is well below 100, and in the Sunderbans it is below 50. Such degrees of infection can be entirely ignored. In many other localities in different parts of the province the index of infection falls below 100, and nowhere in the plains does it reach 200. Only in the Darjeeling district and in the Dooars tea gardens is there any appreciable amount of hookworm. In a village near Kalimpong the index of infection at the season of maximum infection was estimated to be 267, which corresponds with infections in the valleys of Assam. On the Darjeeling tea gardens it appears to be more severe, for on the garden studied, in spite of the fact that 10 per cent of the coolies examined had been treated with thymol within the past year, the index of infection for the season of maximum infection was estimated to be over 400, which is higher than anywhere else in India except in the area described above in Burma. A maximum index of about 400 was found on a tea estate in the Dooars also. In Bihar and Orissa also the incidence of hookworm infections is very high but the degree is low. The egg counts in Bihar were found to be somewhat higher than was anticipated in view of climatic conditions in comparison with Bengal, but Korke (1926) has shown that ancylostomes are relatively much more frequent in Bihar than in Chota Nagpur or Orissa, or more than we have found them in Bengal, in fact he states that *Ancylostoma duodenale* is the predominant species in northern Bihar. This being the case, our egg counts probably indicate fewer worms than do similar counts in Chota Nagpur or Bengal, but since ancylostomes are more injurious than necators, the index of infection derived from the egg counts are more or less directly comparable if considered as an index of the damage done by the infection. The index of infection in Bihar and Orissa lies in general between 75 and 125, and is therefore somewhat lower than that of Bengal.

infestations, 101 to 500 worms, 5, heavy infestations, 501 to 1,000 worms; and 6, very heavy infestations, 1,001 to 3,000 worms. On the basis of the egg counts on mushy Indian stools, these groups would correspond to egg count groups as follows 1, negative, 2, 25 to 300 eggs per gram, 3, 400 to 1,200 per gram, 4, 1,300 to 6,200 eggs per gram, 5, 6,300 to 12,500 eggs per gram, and 6, 12,600 to 37,500 eggs per gram.

Cort et al in China (1926) divided their cases according to degree of infection as follows 1, negative to egg count, 2, 1 to 399 eggs per gram, 3, 400 to 2,999, 4, 3,000 to 9,999, and 5, over 10,000. These counts are on the basis of formed stools, and include a high proportion of ancylostome eggs, therefore they represent much fewer worms than would similar counts in India. To make them roughly comparable with Indian counts it is necessary to divide them by two, for reasons mentioned above it is unnecessary, from a practical standpoint to make any correction for the higher number of eggs produced by ancylostomes. These groups, then, would correspond to Indian egg counts as follows 1 negative, 2, 1 to 200 eggs per gram, 3, 200 to 1,000 eggs per gram, 4, 1,000 to 5,000 eggs per gram and 5, over 5,000 eggs per gram. According to Cort et al, group 2 can be classed with the negatives so far as clinical disease is concerned, group 3 has a slight average reduction in hæmoglobin, and in this group the infection may or may not be significant, in group 4 the majority show a hæmoglobin reduction averaging about 12 points below the negatives, and in group 5 there is clearly severe hookworm disease with an average hæmoglobin reading of about 40 per cent.

Gordon (1925) in West Africa, in a study of 137 natives with various degrees of infections, found that the hookworm infections, 10 of which ranged from 11,200 to 23,100 eggs per gram, produced no noticeable effects on the hæmoglobin percentage, the physique and general fitness, or the mentality of the cases examined, but the figures suggest the possibility of some association between ancylostome infections of more than 15,000 eggs per gram of fæces and the low standard of energy observed in such cases. The individuals studied were youths attending school, city police, and gaol prisoners,—all probably well nourished, healthy, and not overworked individuals, i.e., individuals with every advantage in their favour in the struggle against the effect of the worms, including a possible racial immunity. This work is significant in showing what heavy hookworm infections can be harboured under otherwise favourable conditions without evident injury, but it cannot be applied to the mass of rural Indians.

It will be noted that our grouping is a little different from that of either Smillie or Cort. In some ways it would have been preferable if all investigators had been able to agree on a uniform grouping, but on the other hand it is advantageous, instead of adopting an arbitrary grouping, to divide the cases in accordance with what appears to be a natural grouping, taking into consideration the conditions in the particular country involved.

We feel that we can say with confidence that our groups 1, 2 and 3, with egg counts up to 500 per gram, can be entirely ignored for all practical purposes so far as health is concerned, even in children. A count of 500 eggs per gram in an adult with a necator infection would represent an infection with less than



amount of hookworm is undoubtedly relatively higher, with an index of infection somewhere between 200 and 300, it is, therefore, comparable with the Assam valley

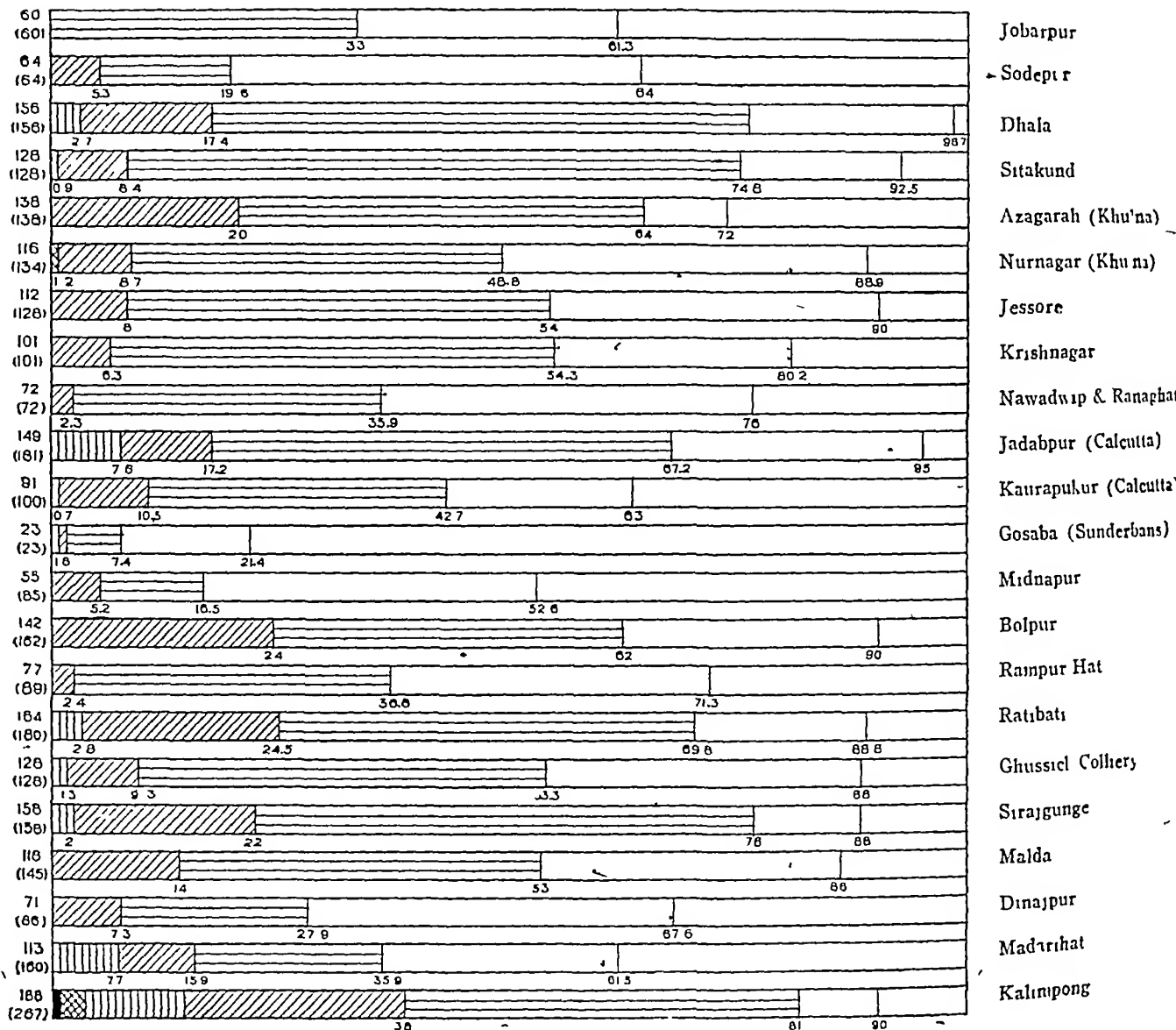
Madras Presidency has within its limits almost every degree of hookworm infection which can be found in India. In the Deccan there is practically no hookworm, in the central part of the peninsula there is a high incidence of very light infections, with indices a little above or below 100, on the east coast north of Nellore, except at the foot of the Eastern Ghats, the index of infection lies between 150 and 200, and is comparable with the infected areas of Bengal, on the east coast from Tanjore to Nellore, at the foot of the Eastern Ghats north of Nellore, and in the south Kanara district on the west coast, the index of infection exceeds 200 and is comparable with the Assam valleys, and on the west coast from the Malabar district to southern Travancore it exceeds 300, particularly on the tea estates. This is about equal to the infection in the most severely infected areas in the Assam valleys, but not as high as in the most severely infected parts of Burma or in the Darjeeling tea gardens

#### BAR DIAGRAMS

The bar diagrams Nos 1 to 9 (Graphs 1 to 9) show graphically the amount of hookworm infection in the various localities studied in each of the divisions of India. Diagram No 10 (Graph 10) shows in a similar way the amount of infection found by other workers in certain other parts of the world. A comparison of this with the Indian diagrams shows at once that only in very few localities in India is the degree of infection equivalent with badly infected areas in other parts of the world. Since the various workers from whose data these foreign graphs were constructed group their infections in different ways, the same shading as was used for the India bar diagrams was not always possible. In every case the egg counts have been reduced to the basis of mushy stools to be comparable with Indian egg counts. Graph A, for Freetown, Sierra Leone, was constructed from egg counts made by Gordon (1925) on school youths, policemen and jail prisoners in that city. Graph B was constructed from egg counts given by Cort, Grant and Stoll et al (1926) for mulberry workers (both sexes combined) in the Ta Chien Meng region of Kiangsu. Graphs C and D were constructed from data based on numbers of worms, given by Smillie and Augustine (1925) for White school children in Alabama. In constructing these two graphs 100 eggs per gram were estimated for every 8 worms. Graphs E and F were constructed from egg count data given by Payne, Cort and Riley (1923) for people living on sugar and coffee estates in Porto Rico. Graph F is for the same group as Graph E, after treatment. It will be noticed that the grade of infection after treatment is higher than in most parts of India without treatment. Graph G was constructed from egg count data given by Davis (1924) for a group of residents of a town of 10,000 population in a rice-growing district in the state of Rio Grande do Sul, Brazil and described by him as 'an area lightly infested with hookworm'. The males in the group were artisans and town workers, not agricultural labourers. Nevertheless, extremely few localities in India, outside of tea gardens, have as much infection.

index number which would serve as a basis for comparison by combining the incidence and degree of infection in such a way as to take account of the distribution of the infections. If all the infected individuals were approximately equally infected we would have only two groups to deal with, the infected and the uninfected, but such a condition is very rarely found. We believe that the distinction between individuals with no hookworms and those with varying numbers from only one or two up to hundreds or thousands is arbitrary and of little value. A distinction between individuals harbouring a number of worms insufficient to produce harmful effects and those harbouring a number sufficient to produce distinct clinical symptoms would be of much greater value. Of still greater value is a series of groups such as those described above, divided according to degrees of infection as indicated by egg counts. If we divided the individuals into only two groups, the uninfected and the infected we could arrive at an index of infection by multiplying the average eggs per gram for the entire community by the percentage of infection, and taking the square root in order to reduce the numbers to a reasonable range. The same result would be obtained by multiplying the average egg counts (i.e. 1/100 of the eggs per gram) of the infected individuals by the square of the percentage and getting the square root. The same principle can be used, and a much more accurate index of infection obtained, by using this method of calculation for each of the arbitrary groups, divided according to degree of infection, and adding the totals to obtain the index of infection for the entire community. For example, if of 100 individuals examined in a community, 20 per cent had infections with less than 100 eggs per gram (average 40), 20 per cent had from 100 to 500 eggs per gram (average 200), 25 per cent had from 600 to 2,000 eggs per gram (average 1,200), and 20 per cent had from 2,100 to 5,000 eggs per gram (average 3,000), the index of infection would be calculated by the following equation  $\sqrt{0.4 \times 20^2} + \sqrt{2 \times 20^2} + \sqrt{12 \times 25^2} + \sqrt{30 \times 20^2} = 13 + 25 + 69 + 110 = 227$ . If the individuals were merely divided into uninfected and infected groups, and the average eggs per gram, which in this example comes to 1,138, were multiplied by the percentage of infection (85), the index of infection obtained by taking the square root of the product would be 310. This would assume that each one of the 85 infected individuals out of the 100 examined has a moderately heavy infection of 1,138 eggs per gram, whereas, as matter of fact, nearly half of the infected individuals have an average of only 145 eggs per gram. The distribution of the infections, therefore, is taken into consideration as well as the total percentage and the degree of infection. It has been suggested to me that a statement of the incidence of infection and the average eggs per gram for the entire community would give a sufficiently accurate indication of the hookworm situation, but the example quoted above shows the fallacy in this. Furthermore, a single index number, if it actually does give a fair basis for comparison, is far more useful than a set of figures which have to be combined in the student's mind in order to make a direct comparison. We have worked out the index of infection as described above for about 150 different localities in India, and we are convinced that the index numbers obtained give a basis for comparison which is as accurate as any single numbers can be as judged by a careful consideration

GRAPH No 3



Amount and Distribution of Hookworm Infection in Various Localities in Bengal  
Shading same as in Graph No 1

The outstanding result of investigations in southern India and of our own in all parts of India is the indisputable demonstration of the fact that, in spite of a very high incidence of infections in some parts of the country, hookworm disease is practically non-existent in most places, and limited to a relatively small percentage of the population even in the most severely affected areas. Only in very few localities in India (some places in Burma, and on some Darjeeling and Dooars tea estates) does the index of infection exceed 400. For comparison, it may be pointed out that in some heavily infected areas in the West Indies, e.g., area C in Porto Rico (Payne, Coit and Riley, 1923), the index of infection approached 600 and in some rural school children in parts of Alabama (Smillie and Augustine, 1925) it exceeds 500.

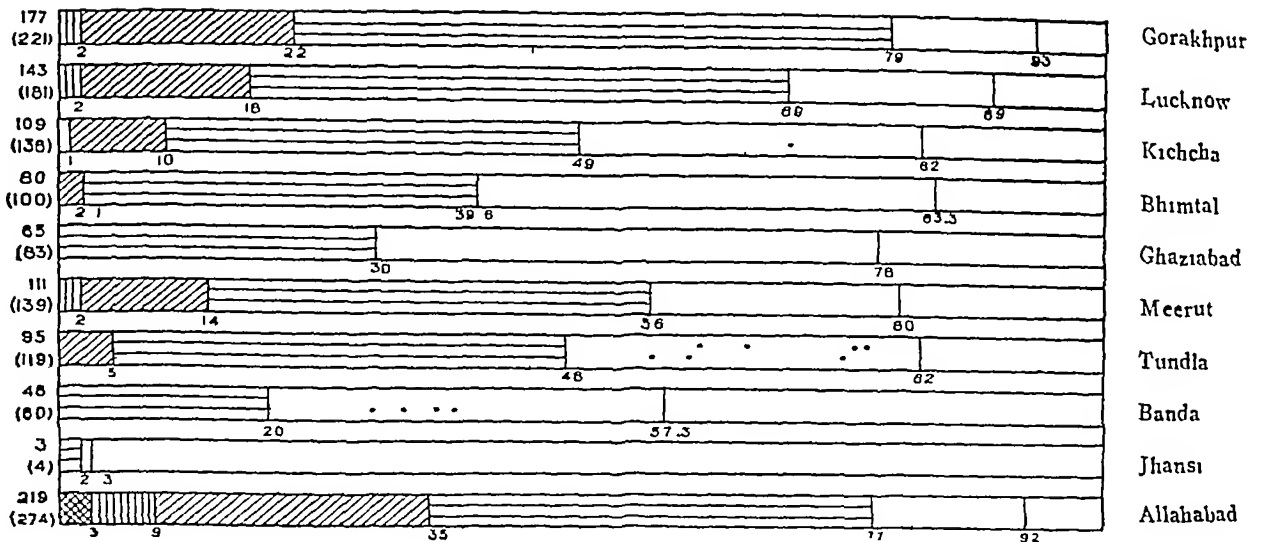
#### BURMA

In Burma the degree of infection varies much more than in any other province except Madras. In the delta region of Burma where the ground is nearly all under water in the rainy season, the use of latrines is very prevalent among the Burmese, though not among the Indians who have settled there, consequently there is a very marked difference between these two races in their degrees of infection. Among the Burmese the average eggs per gram in July varied from about 220 to 460, and the index of infection from 108 to 176. Three per cent or less of them fall into our groups 6 and 7, and from 2 to 3 per cent in group 5. The Indians, on the other hand, and the Burmese who live on the border and between the wet and dry zones, where the ground is not sufficiently flooded to be conducive to the construction of latrines, the average eggs per gram are about 1,380 and the index of infection 440 or more, 5.5 to 8 per cent of them fall in groups 6 and 7 and 12 to 20 per cent in group 5. This is the most severe degree of infection which we have found in any part of India. It is about equivalent to the infection in the mulberry workers in China (the most severely infected group in that country). Rural school children in Covington County, Alabama, according to figures given by Smillie and Augustine (1925), have an index of infection certainly in excess of 500. This maximum Indian infection is also much lower in degree than that found in heavily infected areas in the West Indies which have not been affected by control measures. Fortunately, the intermediate zone in Burma where this relatively high degree of infection occurs is small, and in the delta area it is limited to the relatively small Indian portion of the population. In the dry zone of Burma there is practically no hookworm, and in the northern parts and in the Chin and Shan hills the index of infection (121 to 236) is too low to be of any serious consequence.

#### ASSAM

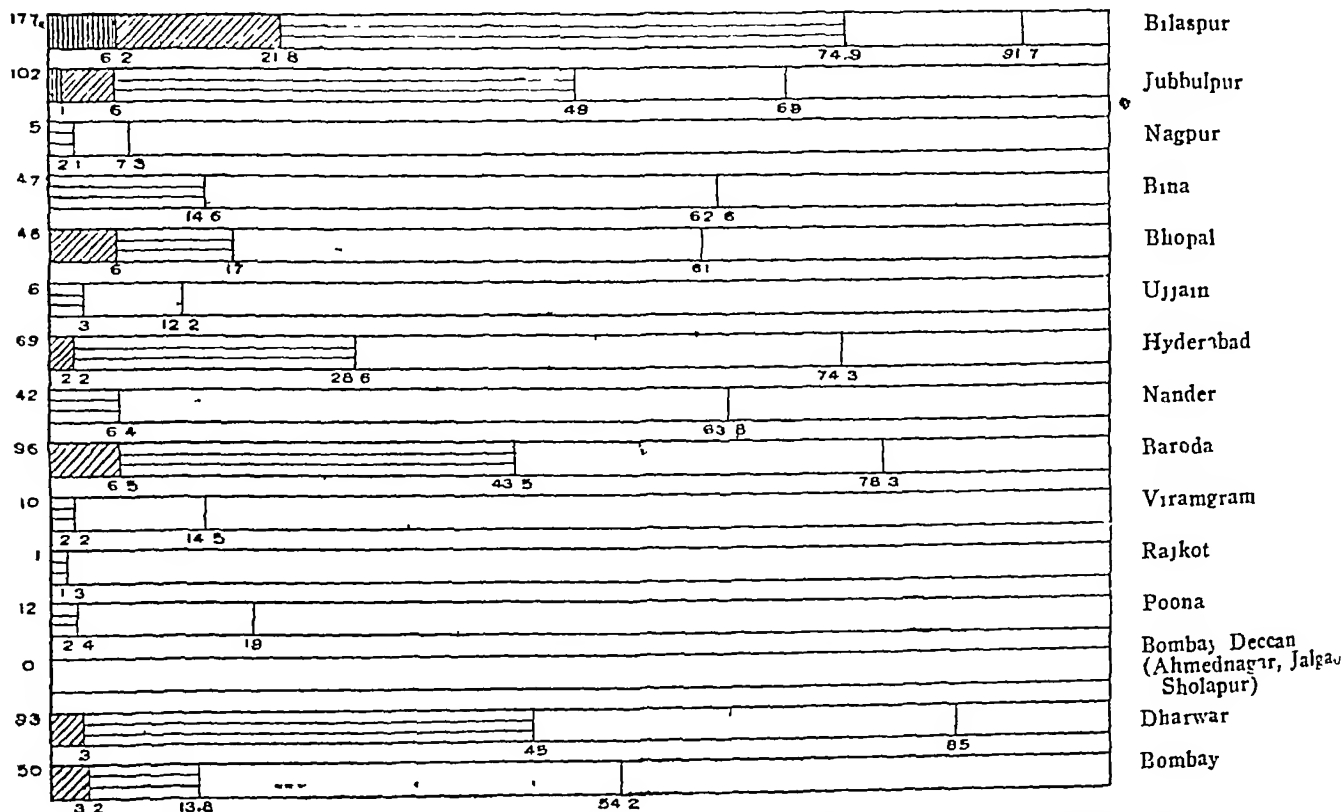
In Assam the degree of infection is fairly high as compared with other parts of India in the Brahmaputra and Surma valleys, particularly on the tea gardens. In the upper Brahmaputra valley the index of infection, corrected for the season of maximum infection, ranges from about 200 to 300, while in the Surma valley it is a little above or below 300. This, however, is considerably lower than

GRAPH No 6



Amount and Distribution of Hookworm Infection in Various Localities in the United Provinces  
Shading same as in Graph No 1

GRAPH No 7



Amount and Distribution of Hookworm Infection in Various Localities in Central and Western India  
Shading same as in Graph No 1

## UNITED PROVINCES AND CENTRAL INDIA

In the United Provinces it is quite probable that ancylostomes are at least as frequent relative to necators as they are in Bihar, otherwise it is improbable that the egg counts would be as high as they are. The index of infection is actually higher in eastern and northern United Provinces than it is in Bengal in spite of less favourable climatic conditions, but, as in the case of Bihar, the number of worms harboured is probably fewer, whereas the severity of the infection is greater on account of the predominance of ancylostomes. The index of infection in the eastern and northern parts of the United Provinces exceeds 200, and gradually decreases towards the south-west. In the Bundelkhand hookworm is almost completely absent, and entirely negligible in the doab between the Ganges and Jumna rivers. A degree of infection comparable with Chota Nagpur occurs in the eastern parts of the Central Provinces, in the rice-growing valleys of Bilaspur and Raipur districts. In other parts of the Central Provinces and in Central India the index of infection is at most a little over 100 (e.g., Jubbulpur) and in most places, especially in the areas with black cotton soil, is far below 100 and entirely negligible.

## NORTH-WESTERN INDIA

The entire north-western part of India (Punjab, Kashmir, North-west Frontier Province, Sind and Rajputana) is for all practical purposes entirely free from hookworm. There is a belt along the eastern and northern borders of the Punjab, including also the eastern part of Rajputana, where there is a fairly high incidence (60 to 70 per cent) of very light infections, but infections severe enough to be of pathogenic significance are practically unknown. Elsewhere in north-west India only sporadic cases of very light infections occur.

## DECCAN AND WESTERN INDIA

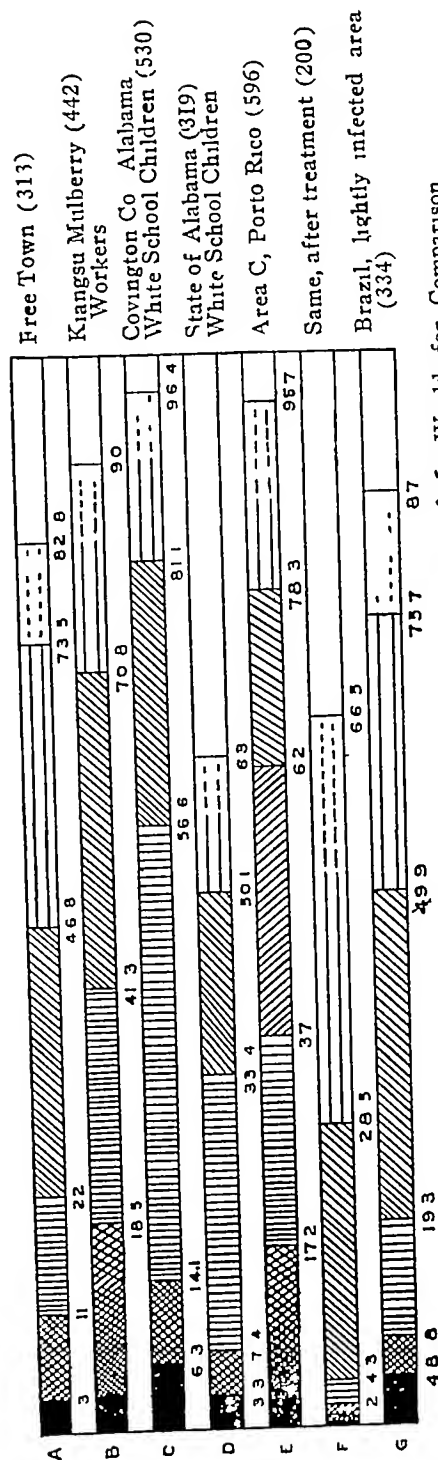
The entire Deccan is practically free from hookworm, especially in the western part, where even extremely light infections are very rare. Nowhere in the Deccan does the index of infection reach 100, such a degree of infection is entirely negligible. Kathnawar and Gujerat are also practically free, although near Baroda, in the path of the northern extremity of the south-east monsoon as it sweeps inland, there is a high incidence of light infection.

The only part of Bombay Presidency which has any appreciable amount of infection is the so-called Konkan, or coastal strip, from Bombay south. Unfortunately we were unable to investigate any villages in this area south of Bombay, but a comparison of the climatic conditions and habits of the people with those on the Malabar Coast of Madras leaves no room for doubt but that a moderate degree of hookworm exists there, with an index of infection somewhere near 200.

## SOUTH INDIA

Mysore is practically free from hookworm, with indices of infection below 100, except the western border of the state, on the crest of the Western Ghats. Here and in Coorg, where there are extensive coffee and other plantations, the

GRAPH No 10



Amount and Distribution of Hookworm Infection in Various Parts of the World, for Comparison with the Indian Graphs

Since the various workers from whose data these graphs have been constructed group their infections in different ways, the same shading as is used in the Indian graphs has not always been possible. The eggs per gram have all been reduced to the basis of mushy stools in order to be directly comparable with the Indian data

In Graphs A and G shading same as in Indian graphs

In Graph B —

Black changing to crossed lines

Vertical lines changing to diagonal

Diagonal lines changing to horizontal

Horizontal lines changing to dots

In Graphs C and D —

Black

Crossed lines

Vertical lines changing to diagonal

Diagonal lines changing to horizontal

Horizontal lines changing to dots

In Graphs E and F —

Black changing to crossed lines

Vertical lines with dotted horizontal lines

Close diagonal lines leaning to left

Wide diagonal lines leaning to right

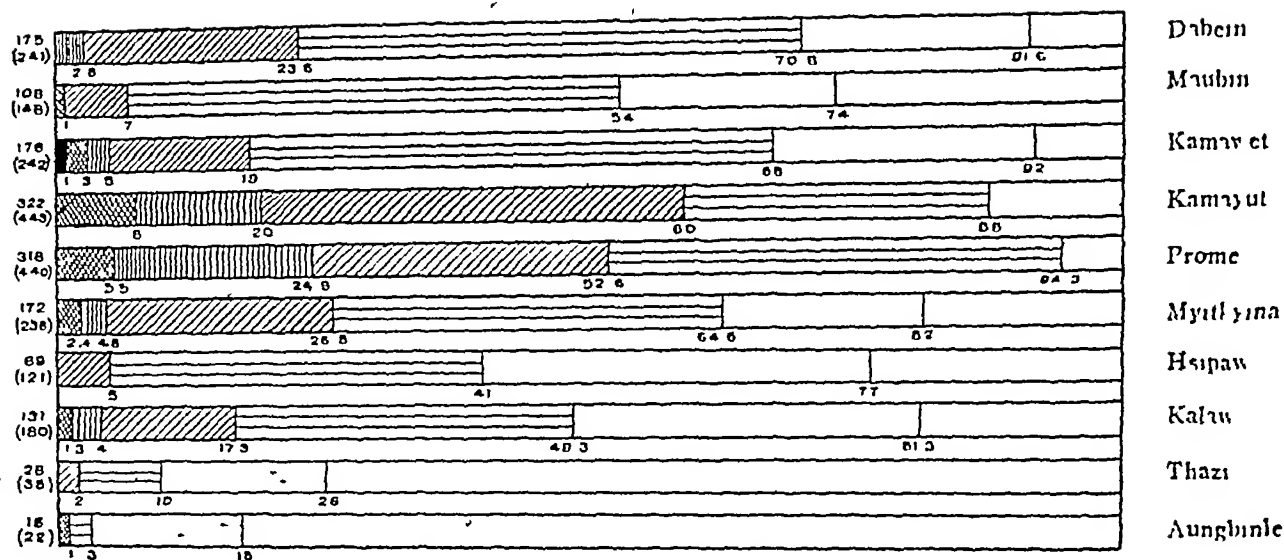
Horizontal lines changing to broken lines

Over 5,000 eggs per gram  
1,500 to 5,000 eggs per gram  
200 to 1,500 eggs per gram  
50 to 200 eggs per gram

Over 12,500 eggs per gram  
6,250 to 12,500 eggs per gram  
1,250 to 6,250 eggs per gram  
300 to 1,250 eggs per gram  
50 to 300 eggs per gram

5,600 or more eggs per gram  
2,500 to 5,500 eggs per gram  
1,100 to 2,400 eggs per gram  
300 to 1,000 eggs per gram  
100 to 300 eggs per gram

GRAPH No. 1

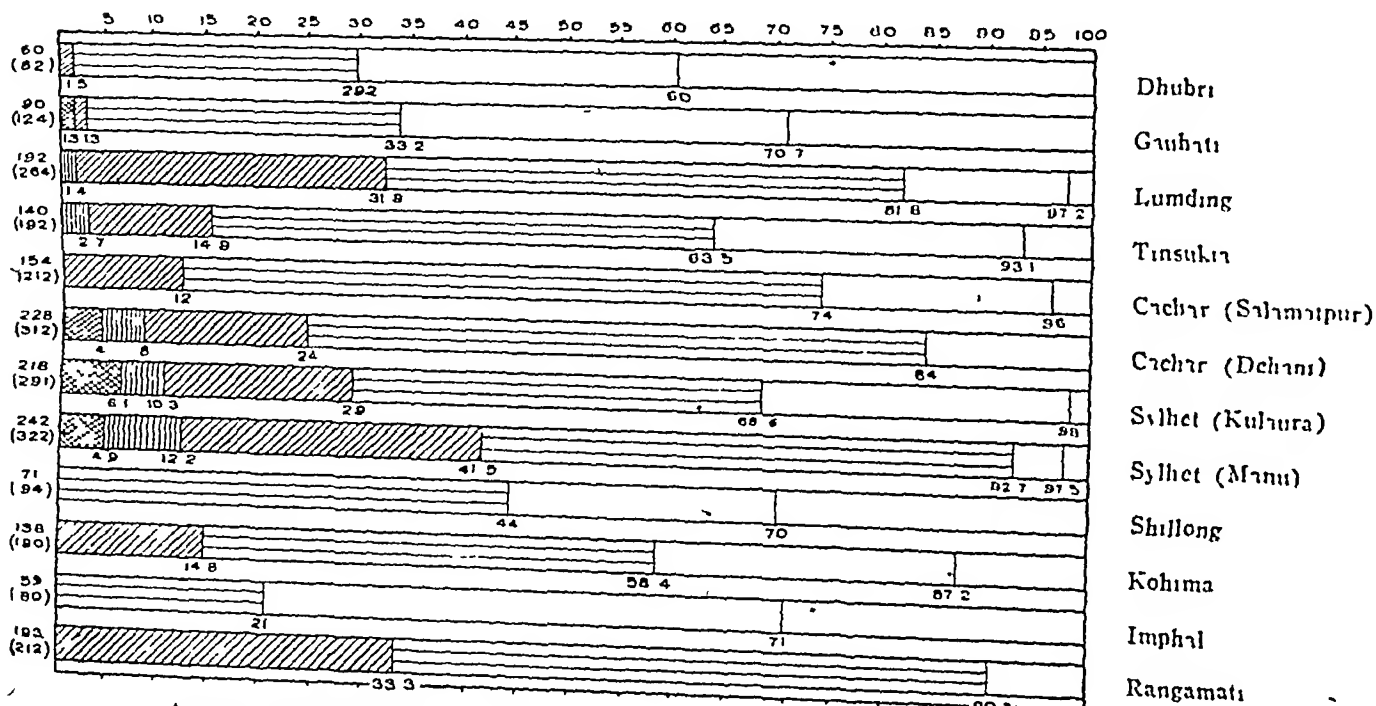


Amount and Distribution of Hookworm Infection in Various Localities in Burma

Solid black Over 10,000 eggs per gram  
 Crossed lines 5,100 to 10,000 eggs per gram  
 Vertical lines 2,100 to 5,000 eggs per gram  
 Diagonal lines 600 to 2,000 eggs per gram  
 Horizontal lines 100 to 500 eggs per gram  
 Dots Under 100 eggs per gram  
 Clear Negative.

Numbers at left designate index of infection at time of investigation, those in parenthesis the index of infection calculated for the season of maximum infection. All egg counts on basis of mushy stools, which are normal in most parts of India.

GRAPH No. 2



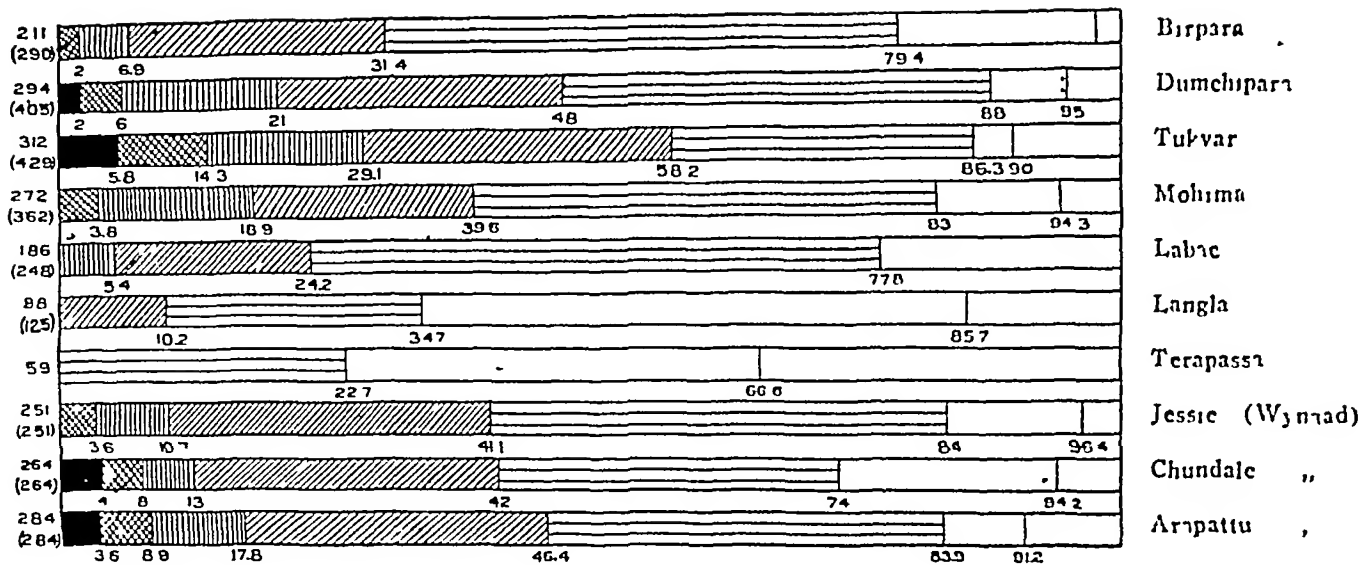
Amount and Distribution of Hookworm Infection in Various Localities in Assam and the Chittagong Hill Tracts  
 Shading same as in Graph No. 1



Trichuris infection is picked up, since the streams do not act as reservoirs or traps in which the worm eggs can accumulate, the eggs are carried away by the flowing water and even the back waters and quiet pools along the sides are flushed out during heavy rains. Some of the Indian tribes, particularly the Santals and some other Chota Nagpur races, are very particular about the source of their drinking water, and never take it from surface tanks or stagnant water courses, they use wells with raised edges in their villages, and when away from home, instead of drinking even from flowing streams they dig holes in the sand or gravel along the sides of the stream and drink the water which filters into it. The Shans in Burma are also very particular. As would be expected, *Ascaris* and *Trichuris* infections are very rare among these people.

As intimated above, there are some instances where prevalent *Ascaris* infections exist without any evident gross pollution of the drinking water. Such localities are always places in which there is a heavy rainfall. Tea garden coolies always have a high incidence and also a high degree of *Ascaris* infections, even when the water supply appears to be above reproach. We have examined large series of tea garden coolies in all the principal tea-growing districts of India—the Assam valley, Cachar, Sylhet, Dooars, Darjeeling district, and the Wynaad of South India, and the incidence of *Ascaris* infections has always exceeded 60 and in most places exceeds 80. *Trichuris* infections are approximately parallel and range from 60 to 95 per cent. On many estates in the Assam valley drinking water is taken from water-filled depressions known as hulas, which are grossly polluted, especially in the dry season when there is no water in them. In the hilly Darjeeling estates, however, there are no ground tanks or pools which could possibly become grossly polluted, for the gardens and coolie lines are situated on steep slopes where only running water is available. In the lines studied on the Tukvar garden, which has 84 per cent *Ascaris* infections and 85 per cent *Trichuris* infections, the water was piped from a mountain spring into an elevated tank from which it was withdrawn from spigots. On one garden in the Dooars water was piped from an unpolluted mountain stream in the Bhutan hills some ten miles distant, and drawn from spigots. In Sylhet the garden studied was supplied with deep 'ring wells' with raised edges, certainly above suspicion of gross pollution. On one garden in the Wynaad area, where there were 73 per cent of *Ascaris* infections, there is a well with raised walls and no tanks or ditches, on another with 75 per cent *Ascaris* infections the water is piped from a distant reservoir, and on another with 61 per cent of *Ascaris* infections there is a well not provided with walls but not obviously subject to pollution. It is difficult to understand this uniformly high incidence of *Ascaris* infections on tea gardens, since it apparently cannot be correlated in many cases with pollution of the drinking water. Some infection can undoubtedly be picked up by washing out the mouth, when bathing with polluted water which is not used for drinking, but this would probably not account for the majority of the infections. There appears to be some factor involved in the transmission of *Ascaris* and *Trichuris* infections on tea gardens which we have so far been unable to identify. A similar condition exists in all places which have very heavy rainfalls, even in hilly places where the ground

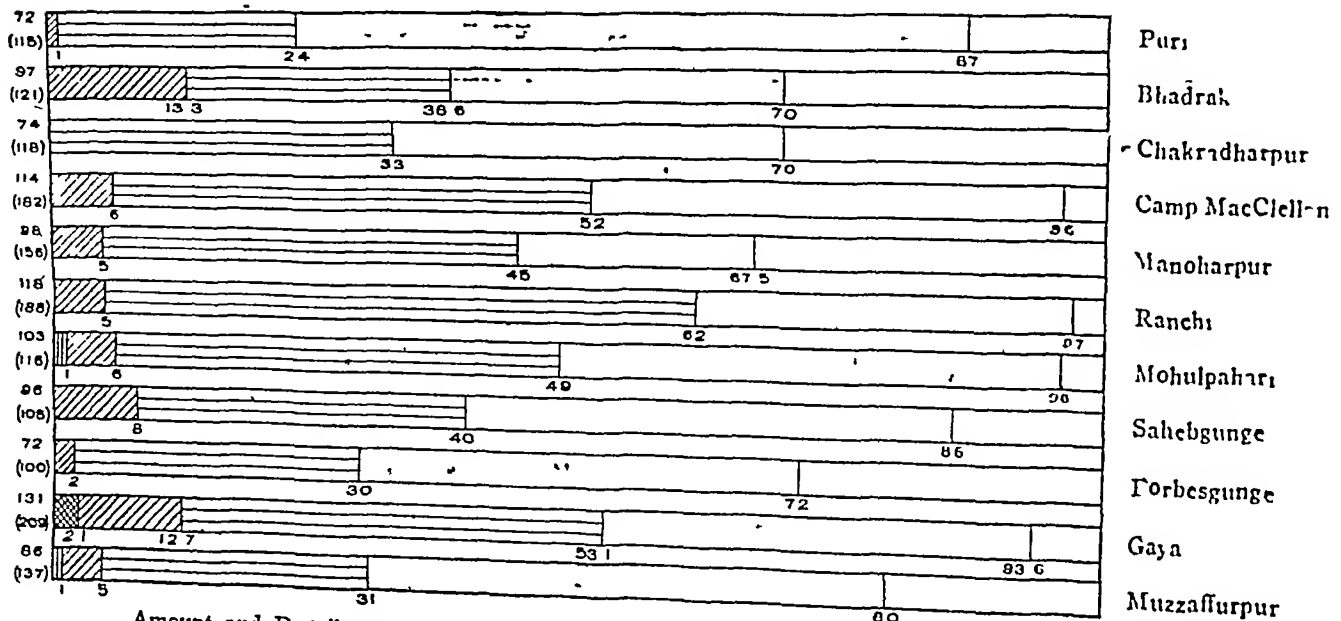
GRAPH No. 4.



Amount and Distribution of Hookworm Infection in Various Localities on Tea Estates in North and South India

Shading same as in Graph No 1

GRAPH No 5



Amount and Distribution of Hookworm Infection in Various Localities in Bihar and Orissa

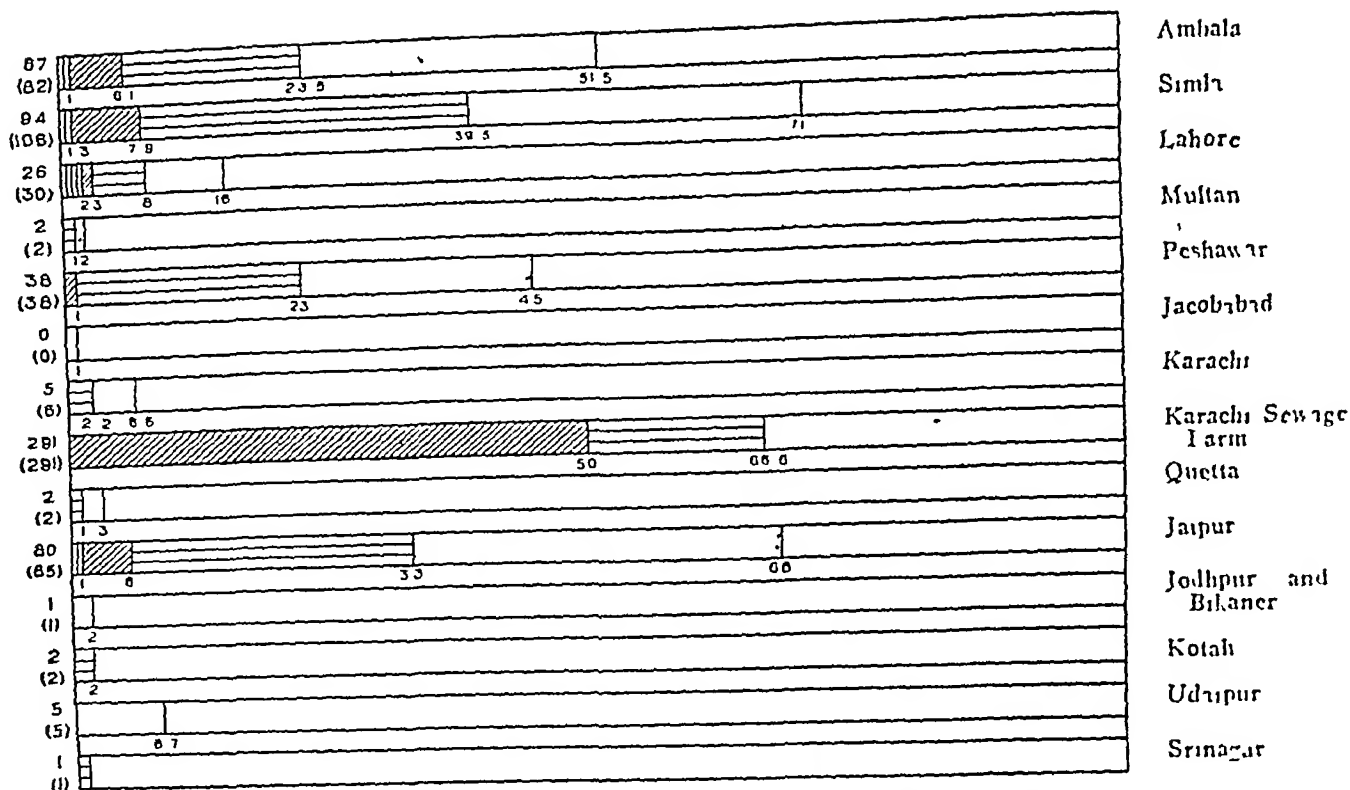
Shading same as in Graph No 1

We have so far considered *Ascaris* and *Trichuris* infections together, on the assumption that both were affected in much the same way by the factors discussed above. The incidences of infection with these two worms are in most cases fairly closely parallel, although *Trichuris* infections, as a rule but not always, are slightly less numerous than *Ascaris* infections. There are, however, a few instances where the two infections are not parallel, indicating that although both are probably primarily dependent on the pollution of drinking water for their transmission, the conditions favouring one may not be favourable for the other. We found only two instances where *Trichuris* infections were strikingly more abundant than *Ascaris* infections. One was in a group of Sauria paharias in the Rajmahal hills of south-eastern Bihar, where 12 per cent of *Trichuris* infections were found and no *Ascaris* infections. These people obtained their water from a mountain spring surrounded by a fence to keep off animals, where gross pollution seemed out of the question. The other instance was among the Manipuris, who had 27.1 per cent *Trichuris* infections and 8.6 per cent *Ascaris* infections. These people use latrines as a rule, and their water is generally obtained from wells free from pollution. We found a number of instances, however, where *Ascaris* infections were out of proportion to *Trichuris* infections. Our observations lead us to believe that *Trichuris* eggs are more susceptible to injury by desiccation than are *Ascaris* eggs, since in dry parts of the country where *Ascaris* infections are comparatively rare, *Trichuris* infections are much rarer still and often entirely absent. Of 5,032 examinations made in dry areas the average *Ascaris* infection was 7.7 per cent and the average *Trichuris* infection 2.4 per cent, which is less than one-third. The most striking instance of excess of *Ascaris* over *Trichuris* infections was in a village near Dharwar in the southern Bombay Deccan. Here 82 per cent of *Ascaris* infections were found and only 1 per cent of *Trichuris* infection. At Hassan on the Mysore plateau there were 57.3 per cent of *Ascaris* infections and no *Trichuris*, and near Bangalore 44.8 per cent of *Ascaris* and no *Trichuris*. Near Ootacamund there were 90.6 per cent of *Ascaris* and only 37.3 per cent of *Trichuris*. These instances clearly point to some epidemiological factor which is necessary for the propagation of *Trichuris* infections which is not necessary for *Ascaris*, but our studies of the local conditions gave us no strong clue as to what this factor might be. Only one possible explanation presented itself at Dharwar and Bangalore, in these places the principal sources of *Ascaris* infections had been done away with within the past two to four years and were no longer operative, it is theoretically possible that *Ascaris* is much longer lived than *Trichuris* and that the majority of the *Ascaris* infections had been acquired several years before, while the *Trichuris* infections acquired at the same time had been lost. Map No. 2 shows the general distribution of *Ascaris* infections in India.

#### OTHER NEMATODE INFECTIONS

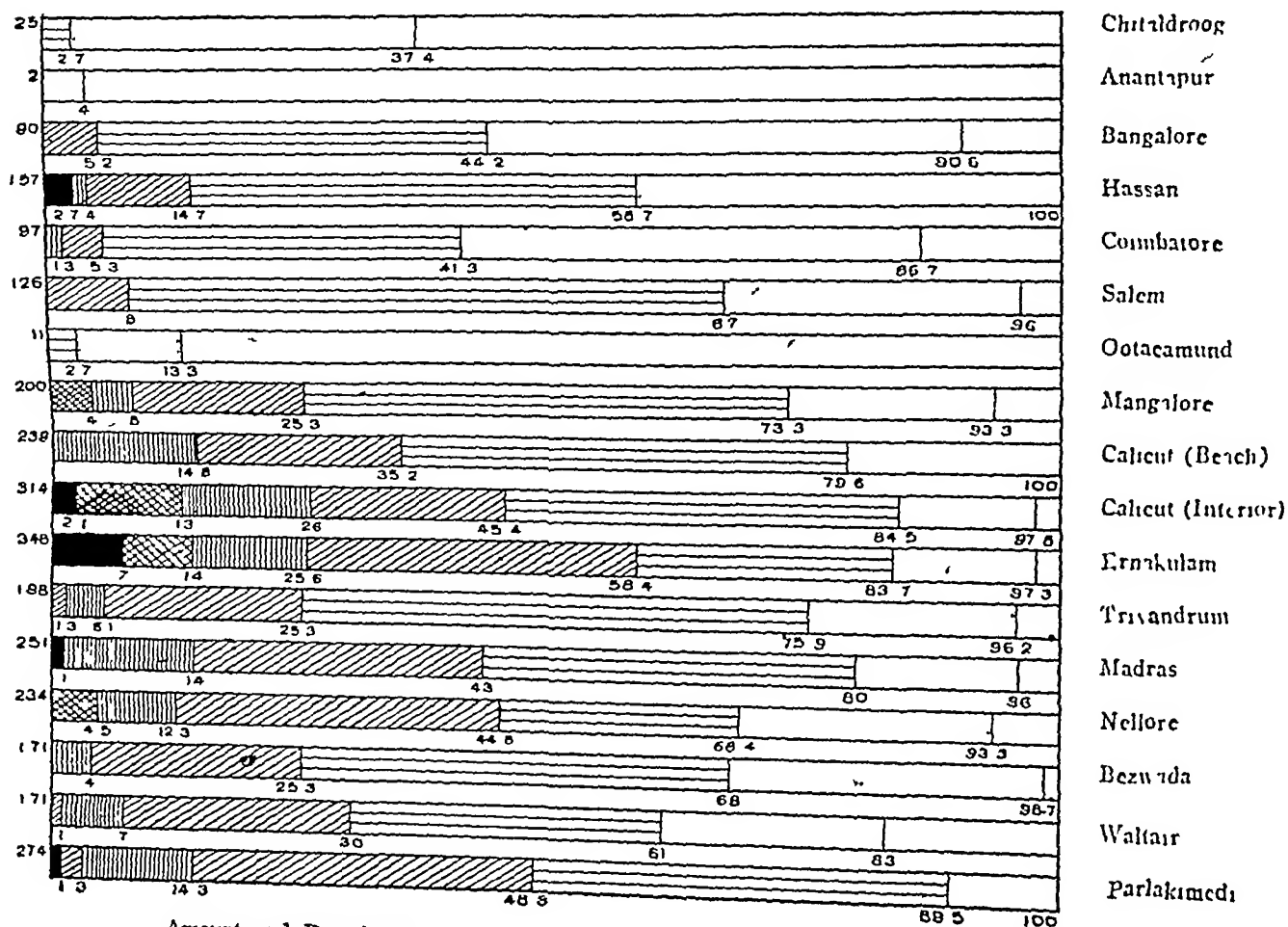
*Trichostrongylus*. In every province in India occasional samples of stools are found which contain *Trichostrongylus* eggs, but their distribution is as a rule very uneven. The most regular occurrence of this infection is in Rajputana and the Punjab, where about one per cent is found in almost every village. It

GRAPH No. 8.



Amount and Distribution of Hookworm Infection in Various Localities in North west India  
Shading same as in Graph No 1.

GRAPH No 9



Amount and Distribution of Hookworm Infection in Various Localities in South India  
Shading same as in Graph No 1.

stage and very few contained embryos. Rhabditiform embryos developed in cultures, but the development did not proceed further to the production of filariform embryos or sexual forms. It is uncertain whether these eggs represent a true human parasite, or belong to a free-living coprophagous species which entered the stools from the soil, but the failure of adult forms to develop in culture makes the latter highly improbable.

*Gnathostomes* Gnathostome eggs were found in human stools on three occasions: once at Kamawet, near Moulmein, once at Aungbingale, near Mandalay in Burma, and once at Errai, near Bhadrak, Orissa. Unfortunately all three of these samples were collected from unidentified stools in defæcation areas near the villages. They had every appearance of being human stools, one contained hookworm, *Ascaris*, *Trichuris* and *Strongyloides* also, one hookworm, *Ascaris* and *Trichuris*, and one only hookworm and *Ascaris*, the latter was found in a much-frequented defæcation area in a village with no pigs. It is within the range of possibilities that these might have been dog stools which strongly resembled human stools, though I think it highly improbable. *Gnathostoma spumigerum* has hitherto been recorded as a human parasite only in an immature state, in subcutaneous cysts, obviously having wandered to an abnormal habitat in an abnormal host, but if able to develop thus far in man it does not appear improbable that they might be able to reach full maturity and produce fertile eggs if they succeeded in reaching their proper location in the walls of the stomach. The infection is very common in cats in Calcutta, and larval cysts are abundant in Bengal snakes. Since snakes are not uncommonly eaten, especially by some Burmese races, there must be plenty of opportunity for human infection to occur.

*Guinea-worm* Our information concerning the distribution and epidemiology of guinea-worm infections has been obtained principally from answers to questionnaires sent to civil surgeons and public health officers in parts of the country where the presence of the worm was suspected. The infection is not endemic, so far as can be ascertained, in Burma, Assam, Bengal, Bihar and Orissa, or the United Provinces, unless in the south-west. In the Punjab there is an endemic focus at the foot of the Himalayas in Hoshiarpur district and neighbouring parts of Kashmir, which probably extends all along the foot of the mountains to the north-west frontier. It occurs locally in nearly all parts of the North West Frontier Province and in the adjoining districts of western Punjab, especially in Attock, Mianwali and Dera Ghazi Khan districts and the northern and western parts of Shahpur district. It also occurs in Hissar district adjoining Bikaner State, and in nearly the whole of Rajputana, especially south-east of a line drawn from the north-western border of Udaipur State to Jaipur. It is common locally throughout nearly all parts of Central India and Bombay except Cutch and Sind, east to about the middle of the Central Provinces, in this area it is commonest in the Bombay Deccan. It is locally common in Hyderabad and Mysore States and in the drier parts of Madras. It appears to be absent from both the west and east coasts of Madras, even where step-wells or ground tanks are in use.

Throughout all the localities where guinea-worm infections occur, it is definitely associated with the use of step-wells (baolis) or tanks as a source of drinking

## ASCARIS AND TRICHURIS INFECTIONS

Our observations indicate that the main source of *Ascaris* and *Trichuris* infections is drinking water which has been grossly polluted by faeces containing eggs of these worms. In most parts of the country there is a very close correlation between the amount of these infections and the extent to which the drinking water is polluted. Freedom from *Ascaris* infections is always associated with freedom of the drinking water from suspicion of gross pollution, though conversely the presence of infection is not always associated with obvious pollution of the water. In parts of the country where the rainfall is light so that the country is not extensively flooded, and where well constructed wells with raised rims serve as the exclusive or at least the principal source of drinking water, *Ascaris* infections are rare or absent, and *Trichuris* infections almost always absent. In Bengal, Assam, Orissa and some parts of Madras, where the rainfall is heavy and surface water abundant during the monsoon season, and where polluted ground tanks are used both for bathing and drinking, both *Ascaris* and *Trichuris* infections are very common. An excellent opportunity to observe the effect of gross pollution of ground tanks used for drinking water was furnished in the flooded parts of Eastern Bengal, where the habitations are built on patches of raised land which to all intents and purposes are islands during the monsoon season. Near Gournadi we found two such 'islands' which were only separated from each other by a canal with raised embankments. One was a small area with 23 households, and a single tank from which all the families obtained drinking water, this tank was so located with reference to the defaecation sites that gross pollution could very rarely if ever occur. Only 3 stool samples (7 per cent) of 66 collected from this locality contained *Ascaris* eggs, and 6 (9.4 per cent) contained *Trichuris* eggs. The other 'island' was a much larger area with over 100 households and with a number of drinking-water tanks. The size of the raised area made it impracticable to have all the defaecation sites located around the margins, and in many places these sites were very near the banks of the tanks, so that large quantities of faecal material would wash into them during rains. Of 35 stool samples examined from this locality no less than 28 (80 per cent) contained *Ascaris* eggs and 12 (34 per cent) contained *Trichuris* eggs. The *Ascaris* eggs were, therefore, seventeen times as frequent on this 'island' as on the smaller one. So far as could be discovered from a very careful investigation of environment, habits and living conditions of the people, the pollution of the drinking water was the only essential difference between them. It would be difficult to plan a more conclusive experiment with respect to the relationship of contaminated drinking water and *Ascaris* infections. Another natural experiment was found at Gosaba in the Sunderbans of Bengal. Here the salinity of water in the canals and most of the tanks entirely prevents its use for drinking, and potable water is only obtained from one tank which is carefully guarded and kept free from gross pollution. Only one *Ascaris* and one *Trichuris* infection was found in 107 examinations made in this place.

When drinking water is taken from rivers or streams, even if these are subject to gross pollution near where the water is taken, much less *Ascaris* and

infection was recorded by Knowles et al (1923) in a Kuki from the Manipur hills near Cachar, but it would appear more probable that this was really an *Opisthorchis* infection. No other indigenous case of *Clonorchis* has ever been recorded in India either in men or animals, whereas *Opisthorchis* infections are very common in cats and dogs in eastern India.

Schistosome infections appear not to have established themselves in India, in spite of frequent importation of the African species by British and Portuguese troops, and of *S. japonicum* by Chinese. Much interest was aroused by the finding of nine cases of *S. japonicum* infection in Chinese at Namtu in the northern Shan States of Burma, but eight of these gave a definite history of residence or visits to Yunan, China, within recent years. We found no cases among native races either in the Shan States or in other parts of Burma, and think it unlikely that the infection has actually become endemic.

Of special interest was the finding of two cases of infection with an unknown schistosome, the eggs of which are different from the described eggs of any known species either in man or animals. It is probably normally parasitic in pigs, goats, or some other domestic animals, and only accidentally parasitic in man. One case occurred in a village near Krishnagar in central Bengal and the other near Kalimpong in the Darjeeling district. This species has provisionally been named *Schistosoma incognitum* (see Chandler, 1926d).

*Hymenolepis nana*. Contrary to the general opinion heretofore, *Hymenolepis nana* is a comparatively common parasite in many parts of India, although there are some parts in which it is almost completely absent. Cases of infection are very rare in Burma except in the dry zone, in all parts of Assam except the Manipur valley, in all but the western tier of districts in Bengal, in Orissa, in Bihar, on the west coast of Bombay and south India, and on the Coromandel Coast. It is much commoner in the central and north-western parts of the country, and approximates 10 per cent in the Punjab and 18 per cent in the Central India Agency. The highest incidence observed in any single locality was among the miners and their families in a colliery in the Asansol mining district, where 21 of 75 individuals examined (28 per cent) harboured this parasite.

In a separate publication (Chandler, 1927) I have called attention to the remarkable relationship of *H. nana* infections to plague on the one hand and to *Ascaris* and *Trichuris* infections on the other. Reference to Maps 2 and 3 will show that the distributions of *H. nana* and *Ascaris* are almost directly opposite, in those localities where *H. nana* infections are common *Ascaris* and *Trichuris* infections are relatively or entirely absent, whereas in localities where *H. nana* infections are rare or absent *Ascaris* and *Trichuris* infections are strikingly prevalent. The indirect ratio is so marked that a suspicion was aroused that the presence of *Ascaris* in the intestine might in itself be prejudicial to the existence of *H. nana*. This, however, does not appear to be so, for in intermediate localities where both *Ascaris* and *Hymenolepis nana* occur in a small percentage of cases, both worms occurred together in one individual in about the proportion of cases which would be expected from a mathematical calculation.

does not become flooded. In lower Burma *Ascaris* and *Trichuris* infections are very prevalent, yet latrines are almost universally used by the Burmese people, and the water is frequently obtained from tank-like wells sunk in the ground. At times of flooding these might become grossly polluted, but their depth is generally sufficient so that the worm eggs would not commonly be withdrawn with the water in buckets, we believe that here as on the tea gardens there is another source of *Ascaris* and *Trichuris* infections which we have so far not been able to discover. The races living in the Assam and Himalaya hills where the rainfall is high are all heavily infected, and the same is true of the people on the wet Karnata and Malabar coasts of south India, the latter commonly use wells which are unprotected by walls but which frequently do not appear to be subject to gross pollution.

We are convinced that flies and dust can be barred as important means of transmission of *Ascaris* and *Trichuris* infections, since in the drier parts of both India and Burma where there is every opportunity for these factors to come into play, *Ascaris* infections and *Trichuris* infections are rare or absent.

Conditions in north-west India suggest that *Ascaris* eggs, and to an even greater extent *Trichuris* eggs, are more susceptible to destruction by desiccation than is commonly believed, at least when exposed to high temperatures, and that a few days' exposure to the blazing sun of these semi-desert countries must be sufficient to kill them. In many parts of the western Punjab, Rajputana, and the North West Frontier Province there are tanks dug, and often lined with brick or cement to catch rain water during the occasional heavy falls in the monsoon season. The surface water flows into these from the surrounding country which slopes towards the tanks, the flow often being facilitated by ditches or small stream beds. No care is taken to prevent pollution of the slopes or stream beds near the tanks, and if the tank happens to be near a village, numerous old and fresh stools can be observed in these places. Much faecal material and any contained *Ascaris* and *Trichuris* eggs must inevitably be washed into the tanks with the rain. Since this water is used for drinking, and in some cases, is the principal source of drinking water for a part of the year, one might reasonably expect to find a fair number of *Ascaris* and *Trichuris* infections. Nevertheless, this is not the case, in a village studied near Bikaner the sole source of water was a tank such as that described above, with abundant pollution all around it, yet no *Ascaris* or *Trichuris* infections were found there. Near Jacobabad in Sind and near Kotah in Rajputana the wells, although with cement sides, did not have raised rims and were subject to pollution by surface washings. The people near Jacobabad sometimes used the water of an irrigation canal also. Nevertheless, no *Ascaris* or *Trichuris* infections were found in these places. If our surmise is correct that the eggs of these worms are destroyed by desiccation in a hot sun at a high temperature, then it would only be the comparatively few stools which had been deposited within a day or two of a heavy rain which would need to be considered as a source of helminthic eggs in the tanks or canals, and the number of eggs thus washed in might easily be insufficient for the permanent propagation of the infections.



*Tænia* Since *tænia* eggs do not occur regularly in the stools, our methods of examination do not demonstrate the true incidence of infections with tape-worms of this genus. Among Hindus *Tænia saginata* is, as would be expected, practically absent, and among Mohammedans it is rare. It occurs in a small percentage of cases, certainly well under 1 per cent, among the lower castes in all parts of the country. We found it to be common only in two races in India, namely the Bhutiyas and Tibetans in northern India, and the Shans in Burma. We found 3 *tænia* infections in 100 stools collected near Kalimpong but about 80 per cent of these stools were from Nepalese and not Bhutiyas. Dr McDonald Smith of the Charteris Hospital, Kalimpong, showed me hospital records of 70 *tænia* infections in 1,400 admissions in one year, almost all of them in Bhutiyas and Tibetans, although not more than about one-fourth of the admissions were people of these races. Our own and Dr Smith's records would indicate at least a 20 per cent infection among the Bhutiyas and Tibetans, and it is probably much higher than this. A dozen random specimens sent by Dr Smith from the Charteris Hospital all proved to be *Tænia saginata*, though it would appear probable that *Tænia solium* is present also. Among the Shans whom we examined in the Shan States in Burma we found about 5 per cent harbouring *Tænia saginata*, both in the northern and the southern Shan States. The actual incidence of the infection is probably greater than this.

Other tape-worms. No other tape-worms were found in our survey, but in the course of our hospital work we discovered a case of infection with *Bertiella satyri*, the second human case on record (Chandler, 1925b). This occurred in a Hindu child resident in Calcutta, who had visited Bihar and Orissa.

### SUGGESTIONS FOR THE FURTHER REDUCTION OF HELMINTHIC INFECTIONS

It is evident from what has already been said that the degree of hookworm infection in most parts of India is not severe enough to warrant any radical measures for its control. Reduction of the infection is, of course, desirable in any place where any appreciable degree of infection occurs, provided this can be obtained without a great outlay of money or time which might be directed against some other public health problem of greater importance.

Reduction in hookworm infection can be brought about either by improvement in sanitary or other conditions leading to the prevention of the acquisition of infection, or by treatment. The former is, of course, the only method which can bring about any permanent improvement, but it is inevitably slow and can sometimes be aided and hastened to a great extent by a judicious use of mass treatments. The latter by themselves can only be looked upon as temporary measures.

### PREVENTION OF PROMISCUOUS SOIL POLLUTION

The ideal to be worked towards in the eradication of hookworm infection is prevention of pollution of the soil, and it can be said with certainty that in no place where climatic conditions are favourable will hookworm infections be

was found fairly regularly in southern India also, where it averages about 0.5 per cent. In all other parts of the country, however, it was too infrequent to be found in most of the localities studied but was common in a few localities. In Bengal and Assam, for instance, *Trichostrongylus* eggs were found only in two places, namely near Midnapore in western Bengal and near Rangamati in the Chittagong Hill Tracts between Bengal and Burma, yet near Rangamati no less than 5 of 21 samples contained eggs of this worm, and near Midnapore the eggs were found in 17 of 97 stools examined. Nine per cent were found near Peshawar, and 14 per cent near Srinagar in Kashmir. It seems to occur quite as frequently among Mohammedans as among Hindus, in spite of the fact that the latter use cow-dung for many purposes for which it is not used by Mohammedans, and might therefore be more exposed to infection by the species which normally live in cattle. We have repeatedly tried to obtain specimens of *Trichostrongylus* from infected individuals by the administration of anthelmintics in order to determine the species, but have not been able to do so. The eggs usually measure between 75 and 90  $\mu$  in length, with a diameter of about 35 to 45. We have not been able to associate the occasional common occurrence of this parasite with any local conditions which might give a clue to its epidemiology or mode of transmission.

*Strongyloides*. Our methods of investigation did not make it possible to determine with any degree of accuracy the prevalence of this infection, since we believe that as a rule the embryos in the stools would die in the containers when kept several days before examination, and also because the D C F method of diagnosis does not bring to light the young embryos. The interesting discovery was made, however, that not infrequently filariform larvæ are demonstrated by the D C F method in stool samples several days old. Of 80 stools from one locality near Khulna, in Bengal, no less than 38 yielded filariform *Strongyloides* larvæ by the D C F method, and of 50 collected near Jessore 7 contained these larvæ. Occasional similar cases have been found in other places, but never such high incidences as these. The evidence from other sources indicates that the true incidence in Orissa is not less than 10 per cent, yet we found only a single infection in 175 examinations in Orissa. Scattered infections were found in Burma, Bengal, Bihar and Orissa and eastern Central Provinces, but none at all in the drier parts of central, northern and western India. We believe that the infection is even more dependent on a heavy rainfall than is hookworm, and thrives best in wet and more or less flooded countries.

An unknown nematode. Of special interest is the occurrence in the stools from some places in southern India of a nematode egg which we cannot assign to any known human species. These eggs were found altogether in 7 stool samples distributed as follows: Dharwar, 1, Anantapur, 3, Salem, 2, and Trivandrum, 1. The eggs, which occur in large numbers in the stools, are 50 to 58  $\mu$  long by 26 to 30  $\mu$  wide, resembling in size and general appearance the eggs of *Rhabditis*. The stools were always several days old when examined and therefore little can be said about the stage of development of these eggs at the time they were passed. Evidently, however, they could not have been in a very advanced stage, for most of them at examination were in the morula

roots of trees, on sloping bamboo poles, on sawed-off bamboo stumps, on edges of pits or ditches with or without bricks or logs to stand on, or on bamboos laid across ditches or pits. In some places it is customary in the wet season to defæcate over the side of a canoe into the water of canals or flooded fields. While most of these modifications of promiscuous soil pollution cannot be considered by any means ideal from a sanitary standpoint, they unquestionably constitute a step forward. If consistently used they very largely eliminate hookworm infection, and the concentration of the defæcation sites, if it does not to a certain extent protect the drinking water source from pollution, at least makes a partial protection more feasible.

There are only two other places in the Indian Empire, so far as we have been able to discover, where the use of any type of latrine is a general custom, namely the Manipur valley of Assam and the flooded areas of Lower Burma. The Manipur latrines are for the most part of very primitive type, usually mere standing places built over or on the edges of ditches or pits, but the Burmese latrines are more elaborate structures with the standing places built well up off the ground, and with protection of the faecal material from domestic animals by means of fencing.

In our opinion the sanitarian can do no better as a start than to encourage the use of these primitive departures from promiscuous soil pollution in the places where they already exist, and by education and exhortation to endeavour to make their use more universal among the children and men as well as the women, and to extend the custom on the borders of the areas where they already exist. The introduction of such customs in places where they are not already in existence appeals to us as having distinct possibilities, education and demonstration would eventually bring results, although a great amount of patience would be required, especially at the beginning. Education concerning the nature and manner of spread of hookworm infections, brought home by suitable demonstrations, and followed by simple and practicable suggestions which would strike at the root of the trouble, could accomplish much more than is generally believed. The average rural Indian will readily grasp the ideas involved if care is taken to put them to him simply and in such a way as to appeal to his reason and prejudices. The most effective way in which the dangers of standing on polluted ground can be brought home to these people is a demonstration of nematodes extracted from soil by the Baermann apparatus, which is simple enough to be included in the equipment of every sanitary worker (*see* Court et al, 1922). After a preliminary explanation in simple terms of the essential facts about hookworm propagation, a sample of moist soil should be taken from a place which was evidently polluted a few days before, such places are generally recognized by the mounds dug up by insects, a fact which is familiar to the villagers. If one or two of the villagers, especially the headman, sees the soil taken and set up in the apparatus, there will be no question of incredulity when results are shown. Next morning, or the same evening, after four or five hours, about 10 c.c. of the water, containing the nematodes, can be drawn off and allowed to settle for half an hour or more. The nematodes can then be withdrawn from the bottom by means of a pipette, placed on a slide,

water, where parts of the body come in contact with the water. In some areas the infection is limited to a very few localities where such water supplies exist, although all such drinking water sources do not serve as foci of infection. The reason unquestionably lies in the absence in some of these baolis and tanks of the particular species of copepods which serve as intermediate hosts, though the reason for their absence is not known. Why the infection should be so definitely limited to the western part of India it is difficult to say.

*Heterodera* Eggs of this free-living nematode were found in stools from all parts of India, usually in not more than one or two per cent of the people examined, but occasionally in over 10 per cent. We do not consider these of any practical importance or significance.

### FLUKES

There has been a suspicion in some quarters that fluke infections of one kind or another would be found to be common in some parts of India. With the single exception of the Manipur valley in Assam, we feel confident in saying that fluke infections do not exist in India except as sporadic cases. In the Manipur valley *Fasciolopsis buski* has long been recognized as being of fairly frequent occurrence, and we found it in 6 per cent of 100 cases examined there. It also occurs sporadically in other parts of Assam but does not appear to be at all prevalent except in the Manipur valley. Sporadic cases of infection with this parasite also occur in Bengal, Bihar and Orissa, and in Madras Presidency. It is very common in Bengal and Assam pigs, which undoubtedly serve as the reservoir for the infection. The life history of this fluke has been shown to be very similar to that of *Fasciola hepatica*, and infection comes from eating water vegetation on which the cercariæ are encysted. In Eastern Bengal, where we discovered three cases, we traced the infection to the eating of water-nuts known locally as 'singara' (*Trapa bicornis*), which are commonly peeled by the teeth. This is interesting in view of the fact that a closely related water plant has been identified as the chief source of infection in China.

*Gastrodiscoides hominis* also occurs sporadically as a human parasite in Assam, though we ourselves did not find any cases of it. Like *Fasciolopsis*, it is a common parasite of pigs in eastern India.

In the Manipur valley we discovered a fairly common pseudo-parasitism with a large fluke of the genus *Isoparorchis*, locally confounded with *Fasciolopsis*, apparently acquired by devouring living flukes with the air-bladder of certain Silurid fishes. The flukes caused a gastro-intestinal disturbance which sent the men to the hospital where the flukes were eliminated after administration of anthelmintics. There was every appearance of true parasitism in these instances. Near Jhansi in the United Provinces we found a case in which fluke eggs measuring 53 to 59  $\mu$  by 34 to 40  $\mu$ , and regularly oval in shape, were being passed. These measurements do not correspond to those of any known human fluke, and we do not know what species was responsible for them.

We have found no endemic cases of *Clonorchis* or *Opisthorchis* in any part of India or Burma, in spite of the fact that *Clonorchis* infections are fairly common in parts of both China and Indo-China. A single case of *Clonorchis*

prevalence of this custom is greatest in the central and western parts of India which are largely unaffected by hookworm on account of climatic conditions, but there are a few places where the wearing of footgear can undoubtedly be credited with preventing a relatively heavy hookworm infection, notably in the eastern part of the United Provinces and in the Shan States of Burma. Even such simple types of footgear as the wooden soles or 'kharams' of the people of the United Provinces must have a very marked effect in controlling the degree of the infection. Where such footgear is already in extensive use, encouragement in extending their use, particularly when visiting defæcation areas, appears decidedly hopeful. It is always easier to make progress with the utilization and extension of an already existing custom than it is to introduce a custom which is foreign to the traditions of the people. In the places mentioned, namely in the eastern United Provinces and the Shan States of Burma, the habit of wearing footgear can be turned to advantage far more easily than the introduction of simple latrines, which are foreign to them, whereas in such places as south-eastern Bengal the situation is reversed. A general use of either habit would by itself, in our estimation, be sufficient to bring the hookworm level down to a negligible point.

On tea estates every effort should be made to prevent the selection of defæcation areas in the gardens themselves, since the cultivated and shaded garden soil is more favourable for hookworm larvæ than the waste land outside, and is a double source of danger, since infection is acquired not only by persons visiting the area for defæcation but also by coolies plucking tea bushes. On most gardens there are drainage ditches already in existence which could be used with good effect as defæcation places. When latrines are constructed for the use of coolies in or near their lines, special efforts should be made to see that they are so built and cared for that they do not become foul and smelly, otherwise the money spent on them is largely wasted.

In mines it is difficult to prevent defæcation in abandoned recesses and passages. As I have pointed out elsewhere (Chandler, 1925) cockroaches constitute an important factor in the control of infection in these situations by devouring the stools and destroying a large proportion of the eggs during their passage through the proventriculus. Suppression of rats in mines helps to encourage roaches.

### MASS TREATMENTS

If sanitary improvements can be developed to prevent re-infection, clinical improvement will, in our opinion, be rapid even if no treatments are given. We have strong experimental and epidemiological evidence for the belief that hookworms harboured are rapidly reduced in number in the absence of re-infections, and we think that within a year all but the most severe infections would be reduced to a practically harmless level, and that most of the moderate infections would reach this point within six months. We have already shown that an annual dry season extending over four to six months has very much the same effect on the hookworm intensity in a community as has a successful treatment campaign. Nevertheless, mass treatments given during the time that

On the other hand there is a striking correlation between the distribution of *H nana* and plague. In all localities where plague is common *H nana* is also common, in localities where plague is rare or absent on account of conditions which are adverse to an abundance of rats, *H nana* is also rare or absent, and in localities where rats are abundant but where special climatic conditions interfere with the development of plague bacilli in fleas, *H nana* is common.

The local distribution of *H nana* infections in India seems to me to throw a flood of light on the epidemiology of the infection. The question as to whether the dwarf tapeworms of rats and of man are identical species, and whether human infection results from ingestion of eggs of rodent or human origin, has long been a matter of dispute among parasitologists. If human infection commonly resulted from contamination of food or water by eggs of human origin, one would expect some correlation between it and the other coprophagous infections, such as *Ascaris* and *Trichuris*, the directly opposite distribution of the two infections is a practically invulnerable argument against the possibility of their having essentially the same mode of transmission. On the other hand the striking correlation of *H nana* infections with an abundance of rats living in close association with man, and the similarity in the distribution of this infection with that of plague when the special climatic conditions affecting the transmission of plague are taken into consideration, point very distinctly to rats as an important epidemiological factor in the distribution of *H nana* infections.

Although the general distribution of *H nana* infections in different parts of India is dependent on the abundance of rats, the more local incidence of the infection in villages varies a great deal. The reason for this local variation appears to be in the habits of the people with respect to the keeping of cooked food overnight to be eaten cold in the morning, especially when the people are careless about keeping it well covered to protect it from rats, would be expected to increase the incidence of *H nana* infections if these are dependent on contamination of food by rat droppings, and our investigations show that this is actually the case. Probably the most careless people in this respect that we have found were the coal miners in the Asansol mining settlement and it is significant that the group of miners studied there had the highest incidence of infection (28 per cent) found anywhere in India.

*Hymenolepis diminuta*. This worm is unquestionably primarily a rodent parasite, and only exceptionally a parasite of man, it has an intermediate host in a variety of insects, including mealworms, which are presumably the usual source of human infection. It is usually considered a very rare human parasite, but in approximately 10,000 examinations made in this survey we have encountered 23 cases, besides a number which we have discovered in the routine hospital work in Calcutta. Twenty-two of the 23 cases were found in places where *H nana* infections exceeded 3 per cent, and only 1 in a place where *H nana* infections fell below 1 per cent. This gives still further support for the theory of the rodent origin of human *H nana* infections. The highest incidence of *H diminuta* which we found was 3 cases among 50 examinations in Asansol town.

treatment for at least another nine months. Provided new coolies are treated as they come a mass treatment once in two years would appear to be sufficient to keep the infection down to a practically harmless level.

The time of year when a mass treatment is given is of very great importance in India, or in any country where there is a dry season sufficient to prevent re-infection. If the treatment is given in this dry season when the ground has been dry long enough to be practically sterile so far as hookworm larvæ are concerned, it is obviously impossible for re-infections to occur immediately from larvæ which have already developed in the soil. On the other hand if the treatment is given when the ground is moist, especially towards the middle or end of the monsoon season, when it has acquired a maximum of infestation with hookworm larvæ, re-infections will occur immediately and, in favourable localities, on a large scale, so that the beneficial effect of the treatment is destroyed almost at once. In all parts of India we consider the most favourable season for mass treatments to be in February and March, though April and May are equally as good in places where rains do not begin until June.

While some consider it preferable, if time and facilities permit, to examine the members of a community before giving mass treatments, in order to exempt uninfected ones from an unnecessary treatment, we doubt if the advantages gained are sufficient to make it worth while. In any community in which the degree of infection is sufficient to justify mass treatments, the percentage of infections will not fall far below 90 per cent and will usually be greater. It is not as easy as one would suppose to get stool samples from all the members of a community, even one as well under control as the coolies on a tea garden or in a coal mine, and to eliminate errors by carelessness or fraud, and the results are therefore not entirely dependable. With certain precautions we do not feel that there is any reasonable expectation of dangerous results from the mass treatments which we recommend, and this does away with the only good argument we can see for not using indiscriminate mass treatments.

For routine mass treatments under field conditions we recommend either a dose of 60 minims of pure carbon tetrachloride, administered in about an ounce of saturated solution of magnesium sulphate, diluted with water if desired, or emulsified in milk followed by the magnesium sulphate, or a combination of carbon tetrachloride and oil of chenopodium. We consider the pure carbon tetrachloride a safe treatment providing the patients are at least moderately well nourished, have normal livers, refrain from the use of alcohol for a day or two before and after the treatment, and are given a diet poor in fats. The general condition of the patients can be determined with a sufficient degree of accuracy by a brief clinical examination, and the restrictions in diet can be insured by a few words of caution and advice, so far as alcohol is concerned, there is an additional safeguard if the treatment is given as far away from pay day as possible.

We believe that a still better treatment, especially if there is any considerable admixture of ankylostomes among the predominant necators, and if *Ascaris* is prevalent, is a combination of 45 minims (about 3 c.c.) of carbon tetrachloride and 15 minims of oil of chenopodium. The chenopodium is best given in hard

done away with entirely until this ideal is reached. In India this is a dream of the far distant future, soil pollution has been the custom in most parts of the country for countless generations, and the difficulties involved in changing it can only be realized by sanitarians who have attempted to do it, even on a small scale. If it is ever to be accomplished, even in part, it is necessary as in any other radical change to keep fighting for it in spite of the apparent hopelessness of the situation at the present time, and in spite of almost complete failure. Here and there results will be obtained, perhaps only on a very small scale, but every such result will be instrumental in bringing about further results, which in the course of time will influence the entire country. Some progress has already been made and more will follow at an accelerating rate.

One cannot hope to jump at once from universal promiscuous soil pollution to completely sanitary disposal of human fæces. The usual procedure on estates is to construct a series of more or less sanitary latrines designed after those in use in European countries, and to demand that the coolies use them. Usually these attempts meet with little success and the manager comes to the conclusion that it is useless to do anything, and that the coolies have inherent prejudices against latrines and an obstinate perverseness which makes them prefer any place except a latrine. As a matter of fact in most cases the trouble lies in the fact that the latrines are so constructed and so used that they very quickly become fouled and so extremely obnoxious that no self-respecting human being could reasonably be expected to use them. True, it is the coolies' own fault that they get in this condition, but carelessness in the use of a latrine is not synonymous with aversion to its use. Often the beginning of the fouling occurs at night or through the carelessness of children. It is difficult for a European who has always used a latrine and instinctively keeps it clean to understand the thoughtlessness of an Indian native who has never used a latrine and perhaps never seen one, and who has no instinctive care as to where he drops his stool. The frequency with which the latrines in railway coaches are fouled by even educated babus leaves no room for wonder that ignorant and undisciplined coolies or villagers should do as badly.

### PRACTICABLE LATRINES

The rural people of India do not like closed-in latrines, with sides and roof, and the development of a general use of them cannot be attained in a single jump. The two principal objections are the almost invariable fouling of the standing places in the latrines, and the disagreeableness of the odour. One can hardly blame an Indian for considering the use of a filthy and smelly latrine far less sanitary and desirable than the use of an apparently clean spot in a secluded place out in the open air. A very much more primitive and simplified type of latrine, consisting merely of an arrangement for keeping the feet from coming into contact with the polluted ground is, it seems to me, a much more hopeful goal for the present. We have found a very extensive use of such a method already developed in certain parts of eastern and central Bengal, where the people habitually stand for defæcation on low horizontal branches or elevated





and demonstrated to the villagers. If only a few people are present, more will come as news of what has been seen is spread. Frequently a slide swarming with hundreds of soil nematodes, with or without hookworm larvæ, will be obtained, but it is sufficient if only a few are obtained. The active wriggling worms make a profound impression, and thereafter there is not only willingness but anxiety to follow any practicable suggestions for keeping these frightful creatures from burrowing through the feet. The fact that some or all of the worms are not hookworm larvæ is of no consequence. If at the same time it has been possible to treat some local individuals and have some expelled *Ascaris* at hand to prove that worms do get into the body, the effect is still better. *Ascaris* always produces a much better effect than actual hookworms.

#### SELECTION AND MODIFICATION OF DEFÆCATION SITES

If attempts to introduce primitive latrines completely fail, some good can sometimes be done by influencing the selection of defæcation sites, or modifying them. Areas which are exposed to direct sunlight, and are covered with heavy clay soil, are less favourable for the development of hookworm larvæ than shaded places and soil which is porous and loamy. We have demonstrated the fact that spreading a layer of six or more inches of old thatch on a defæcation area serves to prevent access of larvæ to the feet of people standing on it until the thatch becomes sodden and mixed with the underlying soil by the activity of insects. This could be prevented by building a brush or bamboo platform six inches over the surface of the ground in order to leave an air space underneath. This would lead to more rapid drying of the thatch or straw and prevent its mixture with earth by insects. In dry weather the fæces desiccate rapidly in such a location, and in wet weather the fæcal material and developing embryos are washed through by rain and come to rest on the ground underneath, where they are harmless. Around concentrated villages where the available defæcation areas are limited and congested it would be difficult to cover a sufficient area adequately to take care of the daily stools, but in villages with scattered and individual defæcation places this method has possibilities.

Another modification of existing defæcation areas is the construction of ditches or trenches on them which can be straddled during defæcation. The demonstrated failure of hookworm larvæ to migrate vertically on a soil wall to any appreciable extent accounts for the success of this method. The larvæ developing from a stool dropped into a trench two feet deep rarely reach the surface on the edges of the ditch. If there is any slope so that the trenches can drain off into a water ditch, and the sides of the trench are plastered with clay to prevent their falling in, they need very little attention.

In some parts of the country, particularly on the Chota Nagpur plateau, there are extensive outcroppings of rocks, and waste lands strewn with stones and boulders. If instead of standing on the ground between or behind the rocks and stones the people could be encouraged to make a practice of standing on the stones or rocks and passing the stools over the edge, there is little reason to doubt that the amount of hookworm infection would be materially reduced.

Another factor which can be utilized to reduce hookworm infection in some parts of the country is the wearing of footgear. It so happens that the

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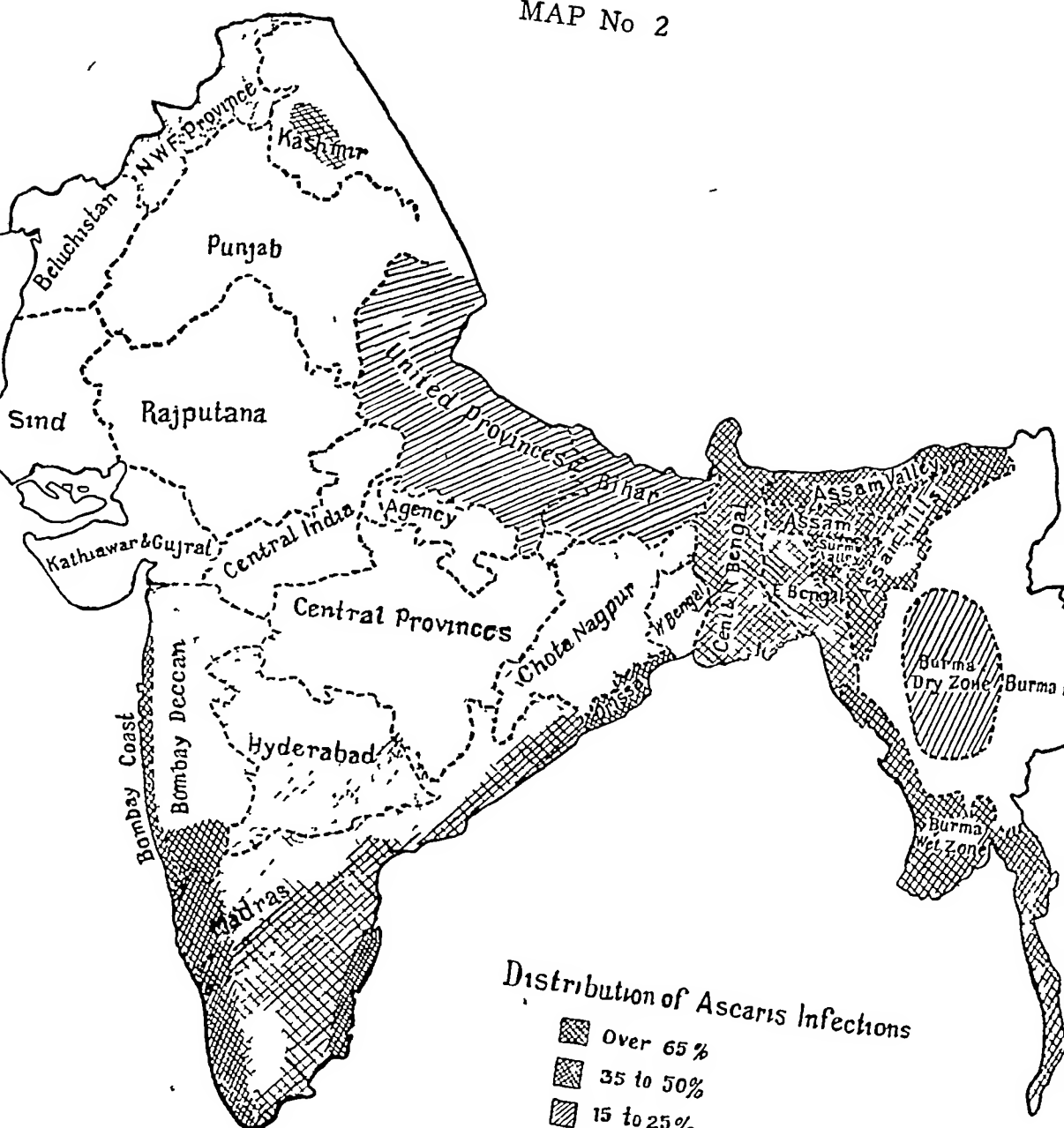
sanitary improvements are being established would unquestionably hasten the beneficial effects, and where practicable are worthy of consideration

When improvement in sanitary conditions on such a scale as largely to prevent re-infection is not immediately possible, but the community to be dealt with is of such a nature as to make mass treatment possible, e.g., coolies on tea, coffee, sugar, or other estates, mine labourers, jute and cotton mill workers etc., such treatments properly carried out are sufficient to reduce the degree of hookworm infection to a practically harmless level, and to keep it there if given at suitable intervals. An excellent demonstration of this has been afforded on tea estates in Sylhet by the work of Rice (1927). We made investigations on the amount of hookworm present on two gardens in Sylhet which had been treated by Rice, all of the coolies on both gardens had been treated in the dry winter season fifteen months before our investigation, and one of them had again been treated in the next dry season only three months before. The treatment consisted of a single dose of 60 minims of carbon tetrachloride in an ounce of saturated solution of magnesium sulphate. All the conditions affecting the degree of hookworm infection on these Sylhet gardens were, so far as a careful investigation disclosed, at least as favourable for a fairly high hookworm index as was present on any Assam or Dooars gardens, and the index of infection in neighbouring native villages was higher than in native villages in the vicinity of any other tea gardens in northern India. We are confident that an average untreated garden in Sylhet would show an index of infection somewhere near 300.






On the garden which had been treated both three months and fifteen months previously (Terapassa) the incidence of infection was 66  $\frac{2}{3}$  per cent, there was an average of only 62 eggs per gram of faeces, and an index of infection of 59. This, it should be remarked, is exclusive of a single individual who had 6,300 eggs per gram, no other individual in the 57 examined had more than 400 eggs per gram. It subsequently developed that this individual had been given the dose with the other coolies both years but had promptly put her finger down her throat and vomited the entire dose both times. On the garden treated only once (Langla), fifteen months previously, and which would therefore show, in comparison with the groups just treated, how much infection was acquired under these conditions, in a year the incidence of infection was 85  $\frac{7}{8}$  per cent, the average eggs per gram were 171, and the index of infection 98. Probably the average eggs per gram at the season of maximum infection would have been in the neighbourhood of 250 and the index of infection about 125. In comparing the once-treated garden with that treated twice, we find that the infections of less than 100 eggs per gram have increased from 36  $\frac{7}{8}$  to 51 per cent, that about 10 per cent have moved up from the 100 to 500 e.p.g. group to the 600 to 2,000 e.p.g. group, and that enough of the very light infections had moved up into the 100 to 500 e.p.g. group to replace those that had moved into the next division.

From an examination of these figures we are forced to the conclusion that, once having reduced the degree of hookworm infection to a low level by mass treatment, it is not necessary to repeat these each year, an infection such as that on the Langla garden, treated once fifteen months previously, does not warrant

MAP No 2



Distribution of Ascaris Infections

-  Over 65 %
-  35 to 50 %
-  15 to 25 %
-  7 to 15 %
-  Under 7 %

Map showing the distribution of Ascaris infections in India

90°

94°

98°

102°

32

# P OF INDIA

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32

## REFERENCE

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gelatine capsules, and carbon tetrachloride as described above, all at one time. Although a little more expensive and a little more laborious than the pure carbon tetrachloride treatment, we believe it is both safer and more effective. The same precautions are indicated as with carbon tetrachloride alone. In either case preliminary starvation is contra-indicated. The treatment should be given about three hours after a moderate meal as near fat-free as possible. Ordinarily the coolies are ready to return to work on the following day, and not more than one half-day need be lost. Exemption from work on the following day should only be given in case there has been unusually severe effects, such as excessive purging, prolonged nausea, etc., and by recommendation of the doctor. Further details concerning these treatments can be found by reference to Chandler and Mukerji, 1925*a* and 1925*b*.

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Probably the Moplahs, who brought the shrub with them from Arabia, were the first to discover the possibilities of coffee cultivation in Coorg and Mysore, and shortly after British rule commenced, the first coffee estate was opened in 1853. By 1864, 70,000 acres of coffee were being cultivated, and by 1878, coffee had reached the crest of its first wave of success. There was a large influx of European coffee planters, and the Kodagas took to coffee growing *en masse*.

About 1883, prices began to fall and the industry received something of a set-back, but nevertheless in 1892, it was recorded that coffee land had never before fetched such high prices nor had coffee planting been more keenly pursued. Such was the pre-occupation with coffee growing that  $1\frac{1}{3}$  of the rice land went out of cultivation, owing to the desertion of the labourers to work on coffee estates, and throughout the country a continuous rise in the standard of living prevailed, owing to the extraordinary activity in the coffee industry and the effect which this had on trade in general.

All classes participated in this rise. It is stated that 'In the earlier years of the decade (1892—1902) almost every Coorg had a coffee estate, or a patch of coffee growing on his own land, the profits from which furnished him with the luxuries of life,—horses, carriages, European liquors and clothing' and it appears that for years the coffee estate was a sort of 'Tom Tiddlers' ground, a source of easy money for all.

In 1895-96, the wave had topped its crest the crops were bad and a fall in prices occurred. In 1900-01, prices fell to such a low level that even a good crop hardly paid for the cost of cultivation, and expenditure on labour, upkeep, manure, and the luxuries of life, fell to a minimum. Large areas of coffee cultivation lapsed into jungle and the acreage under coffee which in 1897-98 had been 85,876 acres, was in 1901-02 reduced to 58,393.

The small cultivator of coffee, the Coorg, for the most part went out of the business altogether, and returned to the cultivation of his neglected ancestral rice fields.

In the decade 1903—12, the depression in the coffee industry reached an even lower level than that of the previous decade. The area under coffee decreased another 24 per cent and all but the best coffee land went out of cultivation, relapsed into jungle, or became overrun with *Lantana*.

The war decade nearly ruined the remains of the coffee industry which was only kept alive by Government loans in 1917, but the last few years have seen somewhat of a revival of its fortunes, and it appears as if the cycle of lean years has now passed.

#### ECONOMIC ASPECTS OF COORG MALARIA

From these facts it may be seen that during the last 70 years, the first half of that period was one of exceptional economic prosperity and the latter half was one of economic disaster and hardship. We would now endeavour to assess, if possible, the degree of influence economic factors may have exerted on that

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- . Investigations on the control of hookworm disease  
XXXIII The significance of egg count data  
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*Hyg*, 4, No 5, 466—500

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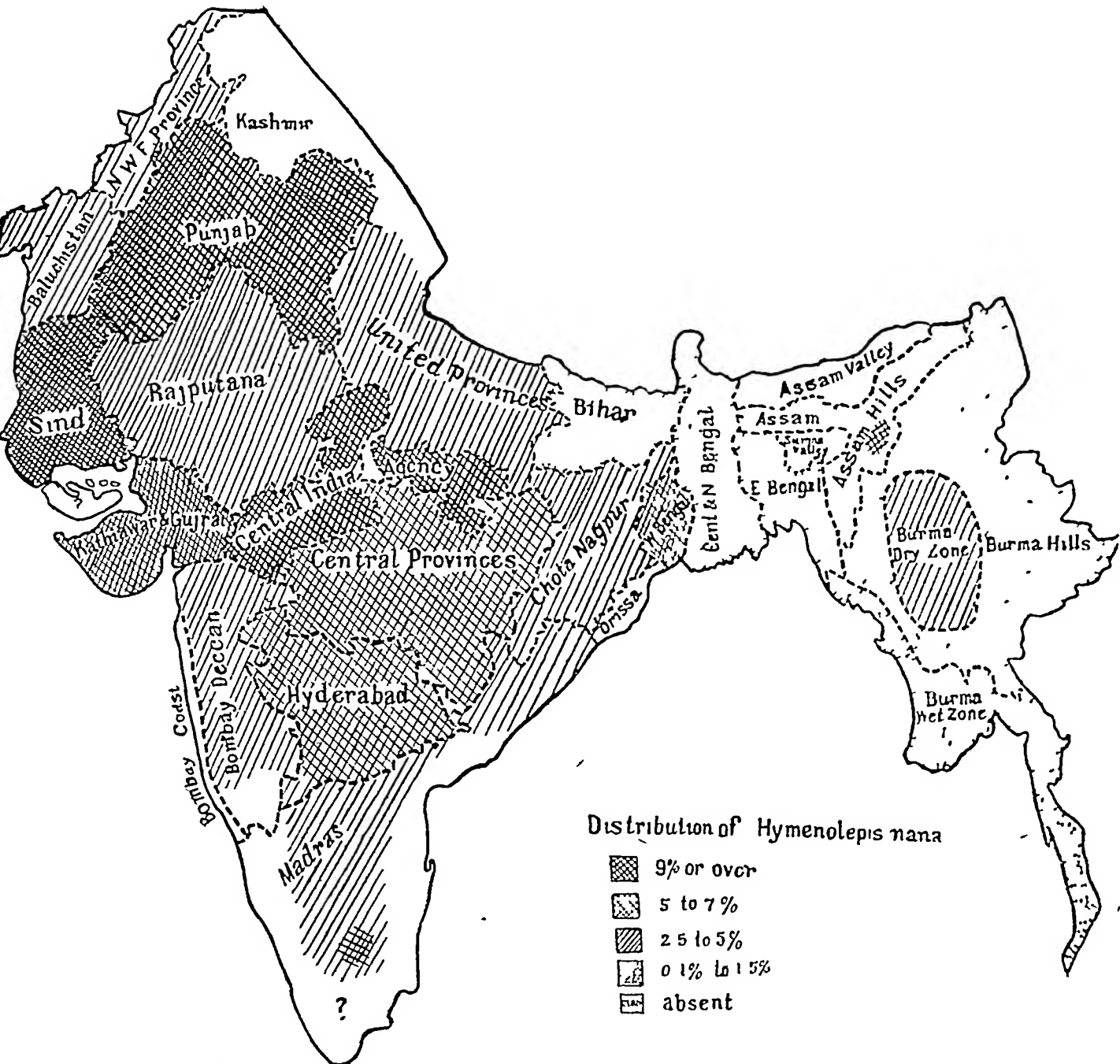
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MAP No 3



Map showing the distribution of *Hymenolepis nana* infections in India

enumeration of an unusually large number of coolies in the census, thus further vitiating any comparison based on total figures

The total figures are therefore of no value as an indication of the variation of the indigenous population

The next step one may take is to examine the fortunes of indigenous races, as revealed by the census returns

The Kodagas, or Coorgs are the dominant race among the autochthones, they were vitally affected by the coffee boom, and by its collapse, and an increase or decrease in their number should be significant

Census years	Kodagas	Variatio.
1871	26,389	
1881	27,033	+ 2.44 per cent.
1891	32,611	+ 20
1901	36,091	+ 10
1911	39,228	+ 8
1921	44,476	+ 13.9

These figures are interesting in that they show a rapid increase up to 1891, while coffee was booming. Unfortunately the 1911 figures are unreliable, for the figures for that year those of another sept, the Jama Coorg, not previously reckoned with the Kodagas, were added. If these numbers, 2,825, are deducted from the Kodaga total for 1911, the +8 per cent variation becomes a decrease, which may very reasonably be attributed to a vital reaction due to the termination in 1895 of the favourable economic circumstances of the coffee boom.

The increase in the intercensal period 1911—21 (if the figures are reliable), would seem to indicate that the Kodagas are recovering from this set-back, but it is noteworthy that the Superintendent of the census of 1921 regards the figures in regard to this caste as too unreliable to warrant this conclusion.

An examination of the fortunes of the next most numerous castes, the aboriginal castes 'Holeyas' and 'Yerawas', shows a decided decrease of late years.

Caste	1891	1901	1911	1921
Holeyas	24,081	26,995	22,951	18,350
Variation (per cent)		+12	-15	-20
Yerawas	14,209	14,585	15,338	14,068
Variation (per cent)		+2.65	+ 5	- 8

# MALARIA IN COORG

BY

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AND

J D BAILY, I M D.

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IN Southern India, on the tops of the Western Ghats, lies Coorg, the smallest Province of British India. Its sixteen hundred square miles of mountain and forest march, on the North and East, with the upland plateau of the State of Mysore, while between them and the sea, at the foot of the Ghats, stretch the lowland districts of Malabar and South Canara. The climate is temperate and humid, the rainfall is intense, vegetation is profuse, and wild animals, birds, and insects are numerous in its abounding forests. Seen from the heights round Mercara, the vista is that of a green sea stretching away to the far horizon, hills and ridges form the waves, the crests and sides of which are thickly clad with forests in whose shade grow the coffee bushes, in the troughs of the waves are patches of the rice cultivation which yields the staple food crop of the Coorg. In the lower tracts round Fraserpet, in the Cauvery Valley, where the country is more open and less hilly, and the rainfall less, the views are those of open grassy glades between avenues of trees and hedgerows.

The inhabitants are distinctive in character, dress, and feature.

The Coorg or Kodaga, the dominant caste, is of good middling stature, fair of skin, pleasant of feature, frank in manner, and progressive and adaptable in disposition.

The lower aboriginal castes, Holeyas, Yerawas, etc., have the usual characteristics of the Dravidian 'untouchable' races of Southern India.

The country was taken over by the British Government in 1832 from a rebellious Raja, since when its history and progress as a self-contained administrative unit, has been determined by the vicissitudes of the coffee industry, which is the chief and only industry of the province. Inasmuch as the history of coffee has been, for the last 70 years, the economic history of Coorg, a brief account of the ups-and-downs of coffee planting is essential to a consideration of Coorg economics, and their bearing on Coorg malariology.

It was not perhaps to be expected that in a period of 35 years of prosperity, which is less than the life-time of a generation, the population would increase to an extent that its pressure on the means of subsistence would give rise to difficulty and economic hardship

The Superintendent of the 1921 census records the opinion that no anxiety as to the existence of such pressure on the available food resources need be entertained, and he remarks that 'The staple food of the country is rice, the outturn is considerably more than is necessary for local consumption, and a large portion is exported at considerable profit'

'The standard of life among the Coorgs is high and the rest of the population are also following suit in the matter of a high standard of comfort, and the labourers, owing to a considerable increase in their wages, have also advanced their standard of living'

The conclusion one would draw from a consideration of these figures is that while the population showed by a 16 per cent increase during the decennium 1881—91 a marked reaction to the period of prosperity in the first half of the 70 years of coffee growing, during the succeeding lean years the reaction has not been a violent one, and a small but regular natural increase was recorded, which received a set-back in the influenza years

As we shall presently have occasion to show, malaria is undoubtedly present throughout the Province, and in such intensity that Coorg must be classed as a hyper-endemic area, but there is no reason to believe that this is a recent development. As long ago as 1870, the Rev G Richter in his Gazetteer of Coorg, refers to 'the dreaded Coorg fever which appears in its worst form especially among Europeans in the vicinity of Alur in S-E Coorg, and about Sampage in the Western boundary'. The Malaria Committee of 1926 comment on the prevalence of malaria in sampage

Recent cases of Blackwater Fever have caused some anxiety in the Province. Lieut-Col Hasell Wright, I M S, in 1920 described several cases of Blackwater Fever in Coorg which had come under his notice, he gave definite evidence of a case as far back as 1901, and he quoted a village headman as stating that such high fevers with dark coloured urine were known to be incurable and were characterised as 'devil's' stroke

Evidently an intensity of malarial prevalence, with occasional cases of Blackwater Fever is no new development

It appears that despite the striking vicissitudes of the chief industry of the country during the last 70 years, contrary to expectation the economic factor has played no appreciable part in increasing or diminishing the prevalence of malaria in Coorg, but whether some exacerbation of an age-long hyper-endemic prevalence has followed the damage which the public health sustained by the influenza epidemic, is a possibility whose exact value is impossible to assess

Some figures put forward by the Coorg Malaria Committee, 1926, in dealing with the 'Extent of Malaria' in the Province (para 20 of their report) have a bearing on this question. They state that 'the hospital statistics (as shown in Appendix No 1) clearly prove the extent of malaria in a progressive scale. In 1918 out of a total of 106,794 patients treated in the hospital and dispensaries,





*List showing the amount and value of Quinine used by the 2 Imperial Government Hospitals of Mercara and Verajpet and the 7 Local Fund Dispensaries in Coorg, during the past 10 years*

Year	IMPERIAL HOSPITAL OF MERCARA AND VERAJPET AMOUNT OF QUININE DRUGS USED				SEVEN LOCAL FUND DISPENSARIES AMOUNT OF QUININE DRUGS USED				Annual Total of Local Fund Dispensaries
	Quinine sulphate	Cinchona febrifuge	Quinine tablets	Total cost	Quinine sulphate	Cinchona febrifuge	Quinine tablets	Total cost	
	lbs	lbs	lbs	Rs As p	lbs	lbs	lbs	Rs As P	
1916 17					86	31		2,118 6 0	lbs 111
1917 18					35½	22		1,167 10 0	57½
1918 19					21½	26		797 2 0	47
1919 20					26½	39		1,929 6 0	65
1920 21					44	36		1,789 6 0	80
1921 22					67	60		3,942 3 0	127
1922 23		72		601 6 0	34	91		2,056 12 0	125
1923 24	80	30		2,496 0 0	42	60		1,735 15 0	102
1924 25	20	280		2,700 0 0	31	215		2,773 14 0	246
1925 26	45	170		2,684 2 0	29	108		1,780 13 0	137
1926 27	105	115	10	3,668 14 0	98	362	46	6,300 8 0	506

aspect of Coorg public health, the prevalence of malaria, which is the subject of this note

It is a truism of economics that in any community, marked reactions are to be expected on the sudden termination of temporarily favourable conditions which have permitted the growth of the population beyond what the country can support when these favourable conditions are at end

From a consideration of the recent economic history of Coorg, the thought arises that perhaps malaria in Coorg may be one of these reactions, and that it may be operating to reduce an inflated population to a level determined by reduction in productivity

The vital statistics of Coorg are not sufficiently accurate to afford us evidence for a discussion of this conception of Coorg malaria, but the census returns, if critically examined, do appear to throw some light on it

*Population of Coorg, decade by decade since 1871*

Census years	Total population
1871	1,68,312
1881	1,78,302
1891	1,73,055
1901	1,80,607
1911	1,74,976
1921	1,63,838

Leaving out of account the census for 1871, which is considered to have been unreliable, since 1881 to 1921 the percentage variation of the population of Coorg was — 8.1 per cent

At first sight these figures seem to support the view that some depopulating influence has been at work since 1901 and that the health of the country, as reflected in the lack of a natural increase, is deteriorating, and those who hold that malaria is on the increase in Coorg find in these figures confirmation of the forebodings aroused by the contemplation of a recorded provincial birth-rate which is greatly exceeded by the recorded death-rate

An examination of these figures in detail shows that these gloomy deductions are unwarranted, immigrant labour to the coffee estates is the distributing factor in the census figures. The labourers on coffee estates are immigrants from South Canara Malabar, and Mysore, who are paid off at the end of a working season that lasts from about August till the end of March, when most of them return to their homes, and dribble back again for another period of work in accordance with the exigencies of the labour requirements in the estates which employ them

When coffee was booming, and the number of immigrant labourers was large, their total naturally swelled the census figures, and *per contra*, in the lean years of coffee, their number was smaller. Another misleading factor is the chance of a late and heavy crop in a census year, which may lead to the

only the merest fraction of rain falls April has, on the average, 2" to 3" of rainfall and May, still dry, has, however, an inch or two more, according as the monsoon happens to break in that month

The temperature is equable, the average mean minimum monthly temperature during November, December, January and February, is below 60°, it falls as low as 56.7° in the coldest month, January, while in the remaining months of the year the average for the month is from 60° to 64.2° The average monthly mean maximum temperature is 85.8°F in the hottest month, which is April, the figures for February and March being 81°F and 84°F respectively During the monsoon months it is 69°F

The humidity is at all times high For 8 or 9 months in the year, the mean relative humidity at 8 hours is about 90 per cent, it more or less approaches saturation in July and August with 96 per cent or 97 per cent, and only during the dry hot months of February, March and April does it in some years fall as low as 65 per cent

These are the meteorological conditions of Mercara An area round Fraserpet in the Cauvery Valley, which is in conformation identical with the Mysore plateau, has a smaller rainfall, some 35" per annum, a less humid climate, and, as we shall see later, a different seasonal incidence of malaria But although in places the rainfall may be slightly less, the climatic conditions of Mercara may be taken as characteristic of the coffee growing uplands of the province in general

The site of the town is a series of ridges On the main ridge stands the Fort, and some of the better class residential quarters and houses, and there are other subsidiary ridges, which are similarly built over Between the ridges lie valleys and streams The valleys are for the most part, uninhabited, as they are seepage areas full of boggy land unsuitable for houses sites An exception to this is Brahman Valley which is less swampy, and has for long been occupied by the houses of the Brahmans connected with the temple, or employed in the offices of the Coorg Administration

The drainage of Brahman Valley joins a stream known as the Fish River whose gathering grounds are springs and seepage areas to the East of the 'Petta'

Some years ago, a survey of the anopheline Fauna of Mercara was carried out by a Civil Surgeon of Coorg, Lieut-Col Hasell Wright, *rms* (1919)

Work in accordance with his recommendations seems to have largely altered the situation in Mercara Many of the unhealthy areas on which he comments have been improved, notably those round Gowli Bazar, and swamps have been drained by putting down open stone-pitched surface drains in them Unfortunately, Hasell Wright's recommendation that covered drains should be used in such work has been overlooked, with unfortunate results

On our arrival in Mercara on the 30th May, 1927, the skies were overcast, the burst of the monsoon was imminent, and it was clear that our time for larval collection would be short

Our energies were immediately directed toward a hunt for larvae, and by separating and going in different directions we just managed to obtain samples

These, however, are jungle people who always tend to recede before the advance of civilisation. All that can be said of them is that they show some increase during the fat years, and have not yet recovered from the effect of the lean years.

Probably the most significant information is afforded by the census table which shows the number born in Coorg and enumerated in the census.

Years	Born in Coorg and enumerated.	Variation.
1881	103,437	
1891	120,110	+16 per cent
1901	125,502	+ 4 "
1911	129,441	+ 4 "
1921	129,901	+ 0.35 "

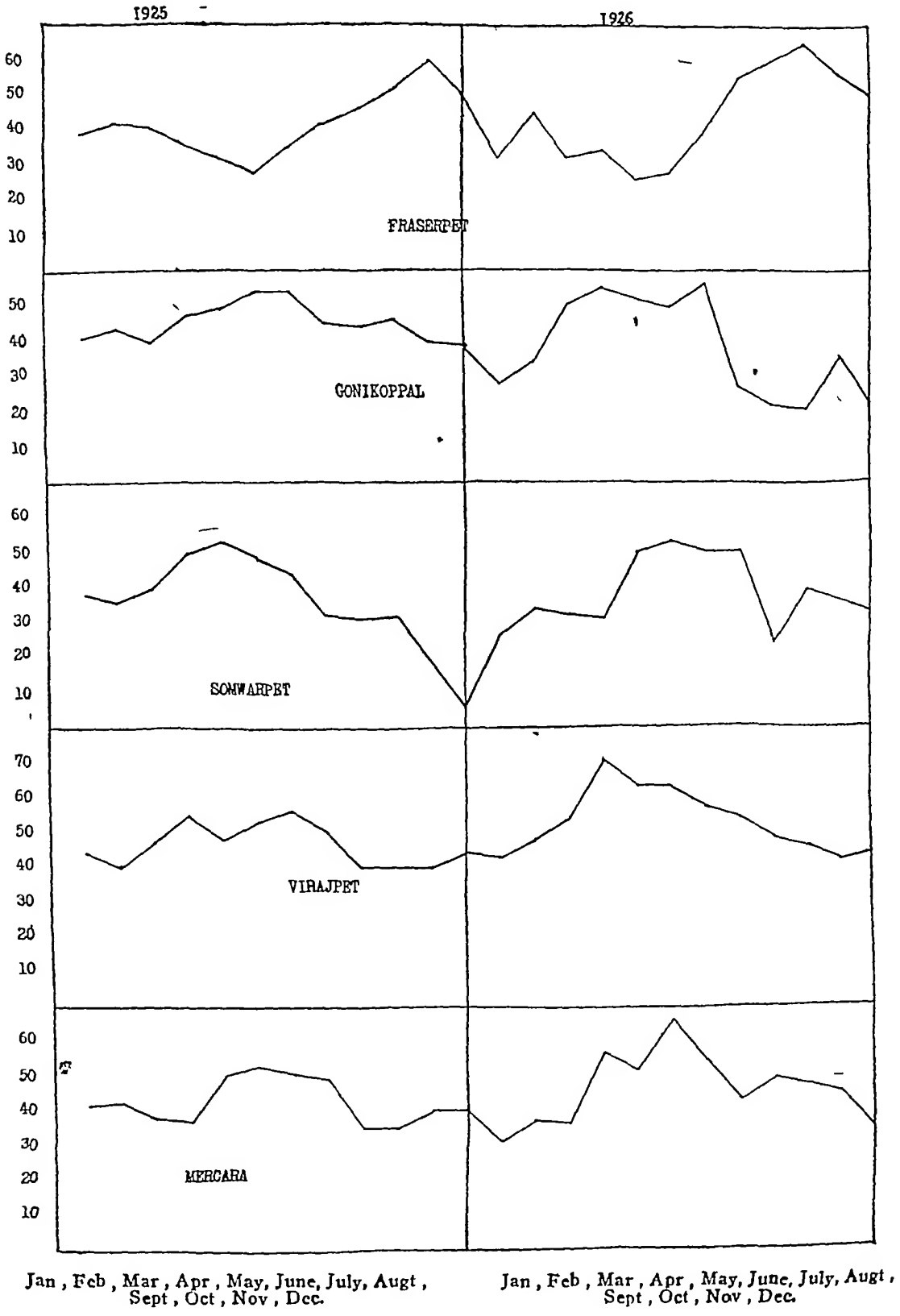
These figures show the enormous increase indicated by a +16 per cent variation during a decade of prosperity, followed by a small but steady increase, +4 per cent, during the years of adversity.

The census population of 1921 records the disastrous effect of the influenza epidemic of 1918-19 and should be viewed accordingly. Turning for the moment to the vital statistics of the province, it appears that the average number of deaths recorded in the three years preceding the influenza epidemic was, in round numbers, about 5,000 per annum. During the years (1918-19-20) which is taken as the period in which the effect of the influenza epidemic was felt, the recorded deaths were 21,673, or 6,420 in excess of the average of the pre-influenza triennium.

Had the 4 per cent increase in population of the previous 20 years been maintained in 1911-21, an addition of about 5,000 would have occurred whereas on the above computation at least 6,000 persons lost their lives during the influenza epidemic (not taking into account those unborn on account of it). The influenza epidemic was, therefore, entirely responsible for the lack of any natural increase in this decennium, and there is nothing to suggest that any other adverse influence on the public health, such as an increase in malaria, has been operative to produce the loss of natural increase in 1911-21.

Similar inferences are indicated by other lines of statistical investigation. Thus, the Committee appointed in Coorg in 1926 to investigate the alleged spread of malaria, refer to a decrease in the population of Mercara (decrease 594) and Verajpet (decrease 646) between 1911 and 1921 and consider that this decrease indicates unhealthiness. The Census Superintendent, however, attributes this to a 'decrease in the number of petty traders and their followers owing to a gradual reduction in the volume of trade as a result of the slump in the coffee industry,' and there is no reason to believe that malaria was concerned with this decrease.

Seasonal Incidence of Fevers



malaria alone accounted for 31,865, or about 30 per cent of the total cases treated. Again, taking the statistics for 1923, out of a total of 152,696 cases treated, malaria alone accounted for 64,269, or a little over 42 per cent which is 12 per cent more than that of 1918. In 1924 we have 90,598 malaria cases out of a total of 187,347 treated, that is, about 48 per cent of the total number treated in the medical institutions of this Province. 'These figures tell their own tale'

The tale at first sight appears to be an alarming one, and seems to warrant the inference that malaria is greatly on the increase, but those who have been connected with rural dispensaries in countries in which malaria is endemic, will suspect a possible fallacy in this inference. The attendance at such dispensaries is often largely determined by the amount of free quinine that can be obtained from them, and an increase in the amount of quinine supplied for use in the dispensary will lead to a proportionate increase in the number of malaria cases attending for treatment.

It is likely that this rule holds good within all probable variations of the quinine supply, as the quinine saturation point of a population living in a hyper-endemic area is to all practical purpose unattainable in charitable dispensary practice.

From the following table, for which we are indebted to the Civil Surgeon of Coorg, Major F. R. Thornton, M.C., I.M.S., it will be seen that omitting the amount of quinine used in the hospitals of Mercara and Verajpet, for which figures prior to 1922-23 are not available, the amount of quinine supplied to the seven local fund dispensaries has of late years been very much larger than it used to be. This increase in the amount of quinine available for distribution from dispensaries may, for the reason given above, be held to account for the increased attendance of malaria patients, to which the Malaria Committee refer.

There is, however, another possible explanation, viz., that the increase of the quinine supply has been made to meet an increasing need for it, and in support of this view one would note that the increase has followed the years of the influenza epidemic, and was not-synchronous with it. This observation would be compatible with the view that an increase in endemic malaria has been provoked by that world-wide catastrophe to the public health.

*Conclusion*—In this connection and at this stage, it may be convenient to summarise one's impression as to the reality, or otherwise of an increase in malarial prevalence in Coorg, which is an opinion generally held there, and one which is by implication the thesis of the Report of the Malaria Committee (1926).

We have seen that malaria is no new disease in Coorg, and that there is no reason to believe that recent adverse economic causes have exacerbated what is probably an age-long endemic prevalence. Nevertheless, our own impression is that there has been some increase, which has been most noticeable in Mercara, owing to the mistaken drainage policy that has been followed there (*see later*).

An impression of this sort with reference to the capital would tend to become one of general application, while at the same time, some additional justification for the opinion would be derived from an increase in the activity of the prevalent endemic disease of the country, as an aftermath of the influenza epidemic.

of active infection, with a rather low degree of immunity, is the state of affairs in Mercara, which to some extent tallies with this popular impression

#### VERAJPET

Verajpet is a rural town of Municipal rank in South Coorg

It is rather more scattered than Mercara, and hill streams, valleys, rice fields and heavy vegetation are the physical features of the town

The monsoon rains prevented all but a very limited sampling of its anopheline fauna

Thus, a hill stream in Gadanga street yielded *A. listoni*, *A. maculipalpis* and *A. jeyporiensis*

Overflow channels from a surface well in block No 5 yielded *A. listoni*

The Raja Kanta tank, a stone-lined surface well at the foot of a ravine, with a drain leading from it to paddy land, yielded *A. culicifacies* and *A. vagus*

A hill stream in Devanga street running in a sandy bed shaded by overgrowth and lined with boulders, yielded *A. listoni*, *A. maculipalpis*, *A. culicifacies* and *A. aitkeni*

A drain in a paddy field yielded *A. listoni*

A surface well full of grass and weed gave *A. culicifacies* and *A. maculipalpis*

A collection from uncultivated paddy land gave *A. jamesi* and *A. culicifacies*

Rocky pools in a stone quarry gave a big hatch of *A. culicifacies* with some *A. vagus* and *A. maculipalpis*

The splenic index here is very high, being 82.8 per cent

The adult spleen rate, as estimated by the examination of 100 persons selected at random in the streets and shops of the bazar, was 58 per cent

The 'fever index' (i.e., the proportion of fever cases attending month by month in the charitable dispensary), indicated the usual spring incident of Coorg (*vide* Chart on page 756)

Many of the 128 children examined for the spleen rate came from outside the town, but there was no appreciable difference between 'town' and 'outside' children in degree of infection

The area is undoubtedly highly malarious, as the above figures indicate, and two notorious carrier anophelines, viz., *A. listoni* and *A. culicifacies*, were identified

Our observations were limited by excessive rain and it is not possible to form any definite conclusion in regard to the malaria of Verajpet, but one may surmise that the hill streams, springs, and seepage areas are the chief sources of its malaria-carrying insect fauna

#### GONIKOPPAL

This is a hamlet in South Coorg where a weekly bazar is held, and a row of shops line the main road. It has a charitable dispensary and a school which serves the neighbourhood

The splenic index is 79.5 per cent and the adult spleen rate as indicated by a small number of observation in the bazar, was 28 per cent

At the same time, it does not appear that the increase has been one of formidable proportions or that it contains any grave and immediate menace to the health of the province

Every country has its own endemic diseases, its own particular thorn in the flesh, to which it owes the greater part of its mortality and morbidity

That of Coorg is malaria, but with suitable local measures (when and where funds permit of them), and with a more general knowledge of the value of measures of personal protection, there seems no reason why a condition of endemic equilibrium and of a reasonable degree of control of the local scourge should not be attained

The succeeding portion of this note attempts to elucidate, in so far as our observations have gone, the conditions and causes of malarial prevalence in certain places in Coorg, and it concludes with such practical deductions and recommendations as can at present be framed \*

#### MERCARA

Mercara is the administrative capital of Coorg. It is a picturesque, green, well wooded, rural town of some 5,600 inhabitants, situated at an elevation of nearly 4,000 feet above sea-level

It has a rainfall of 120" to 130" per annum, which starts about the end of May or beginning of June. Torrents of rain fall during the months of June, July and August, which begin to slacken in September and October, only a few showers fall in November, and from December to March is a dry period, when

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\* The interesting suggestion has been made to the senior writer by a Coorg official that perhaps some connection may be traceable between the prevalence in Coorg of the shrub *Lantana*, and of malaria. It appears that this shrub was imported in 1860, as a fencing plant and, as has happened elsewhere in India, it presently ran wild as an escape. When large areas of coffee went out of cultivation after the slump, this shrub invaded the abandoned land. By 1906, 70,000 acres of private land and 40,000 acres of Government land had been overrun by it, and special measure to cope with its spread were required

It is stated that it has 'encouraged jungle fires, harboured wild animals, rendered homesteads unhealthy, and in particular, that it has reduced the grazing lands for cattle,' moreover, cattle disease has been rife throughout the province and 'milk which could be had for the asking in every village only two or three decades ago has become scarce' (Report on moral and material progress, 1913-27). Especially in the higher coffee-growing lands, the people lament a scarcity of milk and dairy products, which perhaps in the rôle of 'Laudator temporis acti' they considered to be a development of recent years. In support of the truth of this complaint, it may be advanced that the writer has not infrequently noticed rachitic deformities among the children seen by him in the course of splenic examinations in Coorg, which in his experience, is an unusual condition in an Indian rural population, and one which may perhaps be assumed to be due to a dietary deficiency of the anti-rachitic vitamins. There would seem to be reason for suspecting that there is some degree of avitaminosis in the diet of Coorg children in respect of the Fat soluble vitamins, due to a shortage of milk, and dairy products. It is considered by some that such a deficiency renders the subject of it more prone to acquire infections, and presumably, among other such infections, that of malaria. Such, if the argument could be sustained, would be the links between a prevalence of *Lantana* and of malaria. The observation is propounded by the writer more as a matter of interest, than as one of conviction, for the evidence in support of it is slight, and no precedents can be quoted in support of it



would be near their water, as ordinarily it is the proximity of water which determines the site of a village

Doubtless as these extensive floods subside, they leave behind them pools of standing water in which carrier anophelines propagate and produce the autumn wave of malaria which seems characteristic of the Cauvery Valley in Coorg

#### COFFEE ESTATES

Although unfavourable weather greatly hampered our investigation of malarial prevalence on coffee estates, nevertheless, through the kindness of the managers, visits were paid to four coffee estates in North Coorg, viz, Hallery, Jamboor, Coovercolli and Dibidi, and from these visits some information was obtained as to malaria in relation to the coffee industry

As already noted the usual working season is from about August to the end of March, when the labourers are paid off and return to their villages in Mysore, South Canara, or Malabar. Some of them come back about the end of May or June, and a few do not go away at all. Some of them bring their women and children with them, but the number of children on the estates seen by us was small

Men, women and children all work, and are paid by the day. We were informed that the average earnings per day are -|6|-, -|4|-, and -|2|6, respectively. A weekly payment is made, with which the labourers purchase their own food, and bonuses are given for regular work

In case of sickness, no wages are earned, but a weekly advance is given. Pregnant women get, on some estates, a free issue of milk, and are put on light duty. At the end of a working season the labourer's earnings, after deducting advances, is from Rs 20 to Rs 50 per head

The labourers are on an agreement and receive an advance on engagement, but there is no Labour Act nor is there any Government supervision of coffee estates labour

The house accommodation varies somewhat but it is, on the whole, adequate

The usual type of building is a long row of 'lines' on a raised plinth under one roof, with a common verandah, and with separate quarters, two rooms to a 'suite'

The water supply is commonly derived from wells, which are not protected, or supplied with any water-drawing mechanism, or water may be drawn from a stream nearby, or from springs

Latrines are not in use, and adjoining patches of coffee afford the usual shelter for purposes of nature

Ordinarily, the medical treatment of the labour force is in the hands of the estate writer, and no hospital accommodation is commonly provided, but on one of the above estates a hospital, for which a nurse will be employed, is under construction, and on another, a doctor babu, provided with a dispensary, is employed

He states that during April and May about half his cases of sickness are due to malaria. One manager estimated that out of his labour force of some

of the anopheline larvae contained in the different classes of potential breeding grounds in Mercara, before the rains broke on 2nd June, 1927

In a day or two, water channels and pools which had been swarming with larvae were so scoured out that not a single larva could by any means be discovered in them

The disappearance of the anophelines larvae on the burst of the monsoon explains the drop in the malarial attendance at the dispensary some weeks after the onset of the monsoon, and throws some light on the seasonal incidence of Coorg malaria

The subjoined graph of 'Seasonal Incidence of Fevers' shows month by month the percentage proportion of malaria cases among the total outdoor cases attending at five different dispensaries in Coorg

The diagnoses are recorded by Sub-Assistant Surgeons who are provided with microscopes and are more or less competent to use them, and they probably yield a fairly accurate test of seasonal incidence. It will be seen that in Mercara, as elsewhere in the uplands, the incidence of malaria is in the spring months

Our time for precise entomological observation was very short but nevertheless certain useful deductions seem warranted

These may be summarised as follows —

1 In the springs and streams in the seepage areas at the heads of the Brahman Valley in block Nos 17, 18, 19, the stream in block No 21 and from the head waters and tributaries of the Fish River in block No 23, *A. maculipalpis* was the most common species. See map of Mercara

2 Wherever stone-pitched open surface drains had been put down to drain seepage areas, or to carry off household drainage and perennial water, *A. listoni* was found in large numbers, sheltering like trout behind the stones and in the miniature bays provided by the stone-pitched side of the drain. It was by far the most numerous species in the inhabited area, whereas *A. maculipalpis* was the more common species in the heads of the valleys and in the outlying areas under natural conditions

3 In addition to *A. listoni* and *A. maculipalpis*, the following other species were collected —

*A. minimus* was recovered from an up valley drain in Brahman Valley

One specimen of *A. maculatus* was collected in the Brahman Valley drain, down stream

This species was fairly common in Hasell Wright's survey, and presumably its seasonal incidence is other than the time of our observations

*A. culicifacies* was collected in large numbers in a muddy stagnant stone-pitched drain below the Petta, and in muddy hoof-prints in a drain

*A. vagus* and *A. hyrcanus* var. *ingerrimus*, were found in surface wells and springs at the head of the valley

*A. karwari* was seen once in an 'up valley' stream

*A. jeyporiensis*, with some culicine larvae, was found in swampy land

*A. philippinensis* was collected in a tank in an outlying patch of coffee cultivation

It would be well worth while to ascertain why two estates, side by side, should differ so greatly in malaria incidence, for the answer to this question would at the same time be the answer to the question of how to reduce the high figure to a lower level. It is to be regretted that monsoon weather conditions entirely prevented my gathering any information in this connection, and in the interest of the coffee industry. There would appear to be a case for further investigation at a suitable time of year.

One would, however, surmise that the position of the coolie lines with regard to their distance from the ravines, seepage areas, and hill streams in which the carrier anophelines breed, is a very vital factor in the malaria of coffee estates.

#### SEEGAY FIBRE ESTATE

Seegay Fibre Estate is situated about 6 miles from Fraserpet, about the same elevation, 2,800 feet, in the Cauvery Valley. A tributary stream of that river runs past the factory in which the fibre is prepared for export.

The surroundings of the estate are those of the Cauvery Valley round Fraserpet, viz., open pasture land, with trees, and hedgerows and patches of dry cultivation (ragi). The estate itself is situated in the undulating foot-hills which fringe the valley below the heights on which coffee is grown. Adjoining the factory, and some 300 yards from the stream, are the thatched wooden huts in which the labour force of about 40 souls is housed.

Through the kindness of Mr Alderson, the manager, the labour force was mustered for my inspection.

Of these, there were 40 resident coolies, of which 7 were children under ten years of age, of these children, all but one had enlarged spleens, sizes 4, 6, 4, 7, 2 and 4, fingerbreadths respectively.

Of the 35 resident coolies, 21 men and 14 women, 22 had enlarged spleens, or 62.8 per cent and of the 14 who showed no splenic enlargement, two were recently enlisted.

Of eight men (outside labourers), 6 showed a considerable degree of splenic enlargement.

Among 9 outside women labourers of an aboriginal tribe, only two had splenic enlargement, and that to a minor degree, i.e., 1 fingerbreadth. At least two of the resident adult coolies had some degree of pyrexia when examined.

Two springs about  $\frac{1}{4}$  miles from the bungalow from which the water supply is obtained, were examined for mosquito larvae, but none were found in them or in the streamlets which issue from them.

The stream which runs past the factory was examined. This runs through the shades of trees, over a sandy bed lined with smooth rocks, which contain hollows in which storm water lodges.

The stream was very full of tadpoles. The pockets of water in the rocks contained culicine larvae, but a search of the stream, which had recently been scoured out by rain a few days before, yielded only two anopheline larvae, species *A. atkensi*.

A search of the coolie lines, and of the servants' quarters of the Manager's bungalow yielded no adult anophelines, or sandflies. No evidence was obtainable.

Cut banana tree stumps containing water were prolific in culicines but contained no anophelines

Treeholes yielded no anophelines but numerous *Aedes* larvae were found in them

House wells contained no anopheline larvae

The rice fields, in so far as they were examined, yielded no anopheline larvae

House searches for adult anophelines were disappointing in their yield. For instance a forenoon's search in Brahman Valley by two of us, in houses at whose doors *A. listoni* were breeding prolifically, and whose inmates had a high spleen rate, yielded only 3 adults, species *A. listoni*

Such was our general experience of house searches in Coorg, and the explanation would appear to be that in the vicinity of Coorg habitations, the prolific vegetation is a more congenial resting place for the local species of anophelines than are human habitations

No observations on anopheline infectivity were therefore possible

The paucity of anopheline larvae in the marshy land of the swamps and seepage areas of the valleys was significant

Thus, only a single *A. maculipalpis* larva, with numerous culicines, was the result of a prolonged search in the Temple in Brahman Valley

A swamp below the Chaplain's Bungalow yielded a few *A. jaypuriensis*, as also did one below the Wesleyan Church

In a swamp below the Municipal Bazar which Lt-Col Russell, the Director of Public Health of Madras, at a recent visit had considered to be the most dangerous area in Mercara, nothing but culicine larvae could be discovered

From the evidence above detailed, it would appear probable that the anopheline output of swamps in Mercara is insignificant, whereas the output of *A. listoni* from the stone-pitched open surface drains which have been multiplied to drain such swamps, is undoubtedly large

It appears that the policy of draining swamps by open surface drains, which has been followed for years in Mercara as an anti-malaria measure, is a mistake, and that in abolishing a wet area that was, perhaps, doing little harm, the result may have been an increase of malarial prevalence by multiplying the breeding grounds of a notorious malaria carrier

If this suspicion is well founded, it would afford an explanation of the general opinion that Mercara has grown more malarious than it used to be. Hasell Wright (1919) stated that the splenic index of the town was 54 per cent. This agrees with our observations among bazar and school children of from 1 to 10 years of age, which yielded a splenic index of 55.4 per cent, and an adult spleen rate in Mercara Bazar of 53 per cent

There is nothing to show that these figures are comparable, as the age, class, and number of the children, seen by Hasell Wright, and the time of year at which the observation was made, were not stated

In the section of this report which deals with spleen measurements and blood examinations, some evidence is available which suggests that an increase

For those whose mathematical knowledge may be, like that of the writer, scanty, and who may find the above description inadequate for its comprehension, it may be further described as follows

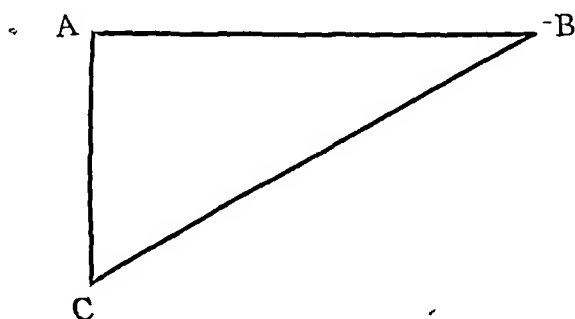
From the filled in chart for each place or series, the totals for each set of measurements are readily and conveniently available

Measurements  $+$  or  $-$  along the horizontal axis are given by the apex-middle line measurement

Measurements  $+$  or  $-$  along the vertical axis have to be obtained by taking the projection of the apex point on to the vertical axis with the umbilicus as 0

This done as follows

In the diagram below, where C = umbilicus, and B = the apex point, the required value is AC, which can be got by squaring BC (the apex-umbilicus measurement), subtracting the square of AB (apex to mid line measurement) and taking the square root of the remainder



In practice, one places in column A, the apex-umbilicus measurement, in B, the apex-mid line measurement, followed by the squares of these columns in C, and D, and their difference, in E. The square root of E is column F. In column G is noted the number of observations for that particular measurement

The figures in columns B and F are multiplied by those in G to give columns H and I

The sum of columns H and I are divided by the sum of column G. The quotient of column H is the mean distance from the middle line, i.e., the position on the horizontal axis (AB of diagram), and that of column I is AC of the diagram, i.e., the apex projection on the middle line or vertical axis

The two values, used as co-ordinates, give the correct position of the mean apex point. The position can be expressed as distance from the umbilicus, and can be entered in the chart, by converting the mean projection back again to apex-umbilicus measurement. This is done by taking the square root of the sum of the squares of the means in columns H and I ( $\sqrt{AB^2 + AC^2}$ ) of the diagram, which give the distance BC of the right-angled triangle ABC, or, the mean distance of the apex from the umbilicus

The subjoined scheme of columns shows what is meant

A	B	C	D	E	F	G	H	I
Apex to umbilicus	Apex to mid line	$A^2$	$B^2$	$(A^2 - B^2)$	$\sqrt{E}$	Obs	See Text.	See Text.

## SOMWARPET

The village is situated in a high ridge, on one side of it is a valley at whose head are drinking water and washing tanks, and at whose lower levels are a few muddy pools and seepage patches

The tanks yielded a few *A philippinensis* and the muddy pools *A vagus*

At the other side of the hill is a valley in which runs a hill stream joined by brooks and drainage ditches

The stream, which had recently been scoured out by the first burst of the monsoon, yielded a scanty catch of *A histon* and *A minimus* and one of its tributary brooks yielded *A tessellatus* and *A antkeni*. Under dry weather conditions this stream probably yields a plentiful supply of *A histon* and *A minimus*, sufficient to propagate malaria in the village on the ridge above it

The splenic index, as measured by an examination of 103 children was 49.5 per cent and out of 30 adults examined in the bazar, 14 had enlarged spleens or 46.5 per cent

The seasonal incidence is, as elsewhere in the highlands of Coorg, a spring incidence (*vide* Chart on page 756)

## FRASERPET

This village lies in the Cauvery Valley at an elevation of about 2,800 feet

Unlike the coffee growing uplands of Coorg, the scenery is open, and the country more or less flat

When Mercara is wreathed in monsoon cloud and drenched with rain, as often as not the sun shines in Fraserpet, and its rainfall is by comparison but an occasional shower or two, being only about one-quarter that of Mercara

An examination of its dispensary statistics reveals the fact that its malaria season is in the autumn, and not in the spring as it is in the uplands of Coorg

Except for the Cauvery river, running rapidly in a deep cut bed, there is no permanent water, the staple food crop is 'ragi' and there are no seepage areas and moist valleys in which rice is cultivated

Its wells do not breed *A stephensi* as had been suggested by others

At the time of our visit, potential breeding grounds were almost absent. A pool of recent water of some weeks' standing, yielded a very few larvæ of *A vagus* and *A maculipalpis*

Adult mosquitoes could nowhere be found, and a single specimen of *A vagus* was the sole yield of a morning search of the bazar

Yet the splenic index among 113 children was 64.6 per cent and among 56 adults examined by random selection in the bazar, 48.2 per cent had enlarged spleens

Of the 18 children under ten years attending the village school in Mullusoga, a village about a mile from Fraserpet, all but one had enlarged spleens

The explanation of the malaria of Fraserpet is probably to be found in the flooding of the country by overflow from the Cauvery river about the end of July. These floods are evidently extensive, as the villages in this area are all situated on high ground, and, it may be, at a considerable distance from the river, their source of water supply. Obviously, were it not for flooding, they

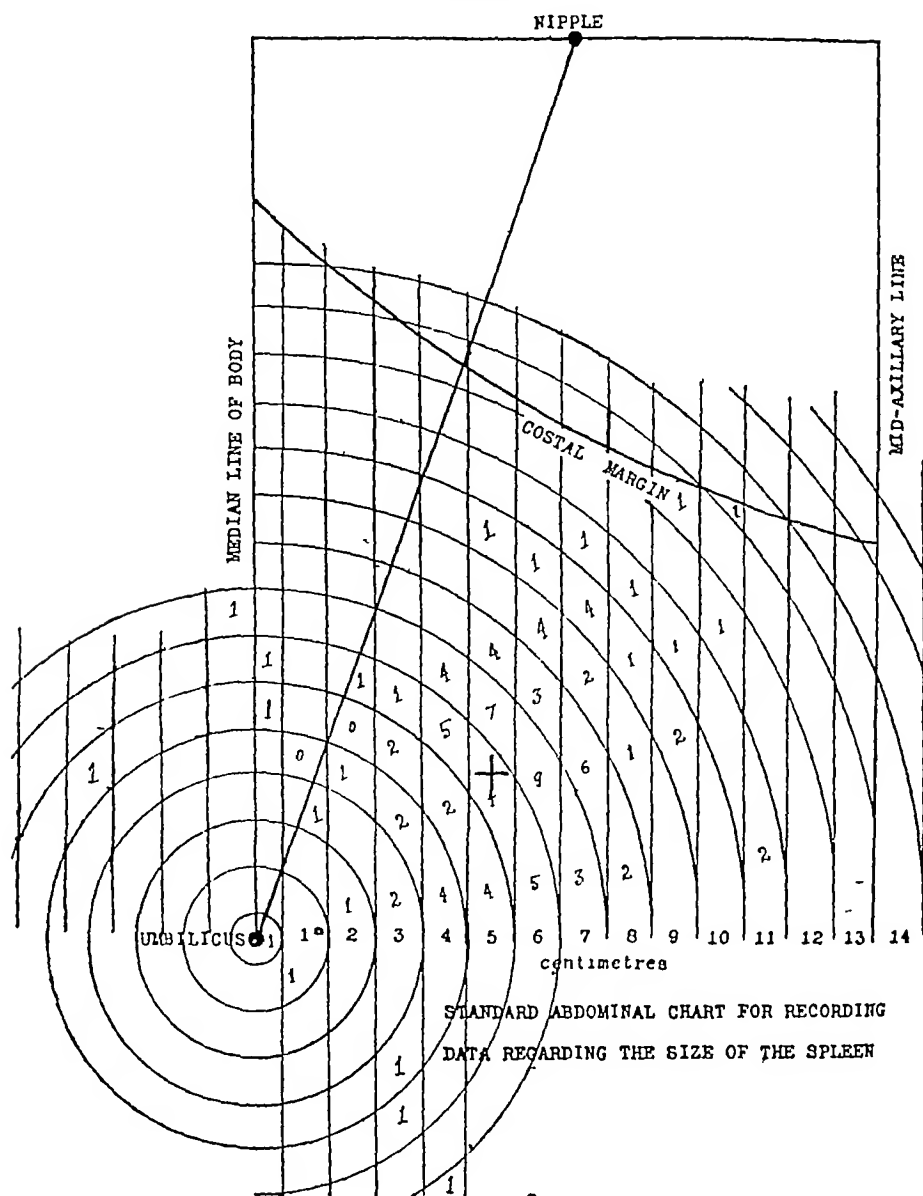
The average infantile spleen of Mercara lay with its apex in square 7|6, 7.1 cms from the umbilicus, 4.4 cms above it and 5.6 cms from the middle line

## II VERAJPET

In Verajpet in South Coorg, 128 children were examined of whom 106 had splenic enlargement, yielding a splenic index of 83.8 per cent

The corrected average infantile spleen had a costal projection of 6.8 cms from the costal margin

### *Verajpet*



The average adult spleen among 100 adults examined was 3 fingerbreadths, or 6 cms which when corrected to standard size would be 5 cms

The apex of the corrected infantile spleen lay in square 6|5, being 6.3 cms from the umbilicus, and 3.5 cms above it, and 5.1 cms from the middle line

300 persons, during the busy season, about 10 to 12 persons per day would be absent, suffering from malaria, but no definite statistics are maintained from which a mere definite statement of labour inefficiency due to malaria can be formulated.

Judging from the physical appearance of the labourers, ankylostomiasis must be very prevalent and many managers find it advantageous to dose their labour force twice a year with carbon tetrachloride.

In general, the hygiene and economic conditions of the labour force of the coffee industry compare unfavourably with those of the Assam Tea Industry, with which the writer was at one time familiar.

The reasons, however, are obvious. The Assam planter has to import his labour at considerable expense to himself from distant areas, and the *per capita* cost of a tea garden labourer used to be estimated at Rs. 300.

The Coorg coffee labourer comes at his own expense from adjoining districts, much less expense is incurred in recruitment, and speaking generally, the supply automatically adjusts itself to the demand.

Tea, moreover, has been for long a large and flourishing industry whereas coffee has been, as we have seen, for 35 years in low water.

One has to recognise that the hygiene and management of a labour force in the East is primarily a business proposition, and is not by any manner of means a philanthropic measure.

The Coorg coffee planter has not for years been in a position to spend money on his labour force, and fortunately for him, the economic conditions and those of the local labour market, have not rendered such expenditure imperative. There is, however, reason for thinking that as a business proposition, some improvement might profitably be effected in the conditions of life of the labour force, now that the industry is recovering its prosperity.

Such could be effected on the lines of estate labour hygiene elsewhere, by the provision of hospitals and expert medical attendance, by providing protected water supplies, and by making a beginning at some sort of conservancy, e.g., by providing pit latrines.

Malarial prevalence and how it may be ameliorated is, however, the particular aspect of this question with which we are at present concerned, and perhaps the only significant observation which emerged from an examination of the labour force in these four estates, was the marked difference in the spleen rate.

Thus, Hallery had a spleen rate of 50 per cent among the 16 resident children and 46 per cent among 53 resident adult labourers.

Jamboor had a 100 per cent spleen rate among 20 children and 91 per cent among 23 resident labourers.

Coovercolli had a 38 per cent spleen rate among 21 children and 33 per cent among 64 resident adult labourers.

Dibidi had 100 per cent spleen rate among 10 children,—all that the estate could produce,—and 73 per cent among 37 resident adult labourers.

Coovercolli and Dibidi are adjoining estates. In the former case, the coolie lines are on the top of a hill a long way from the ravines at its foot, while those of Dibidi are nearer the stream.

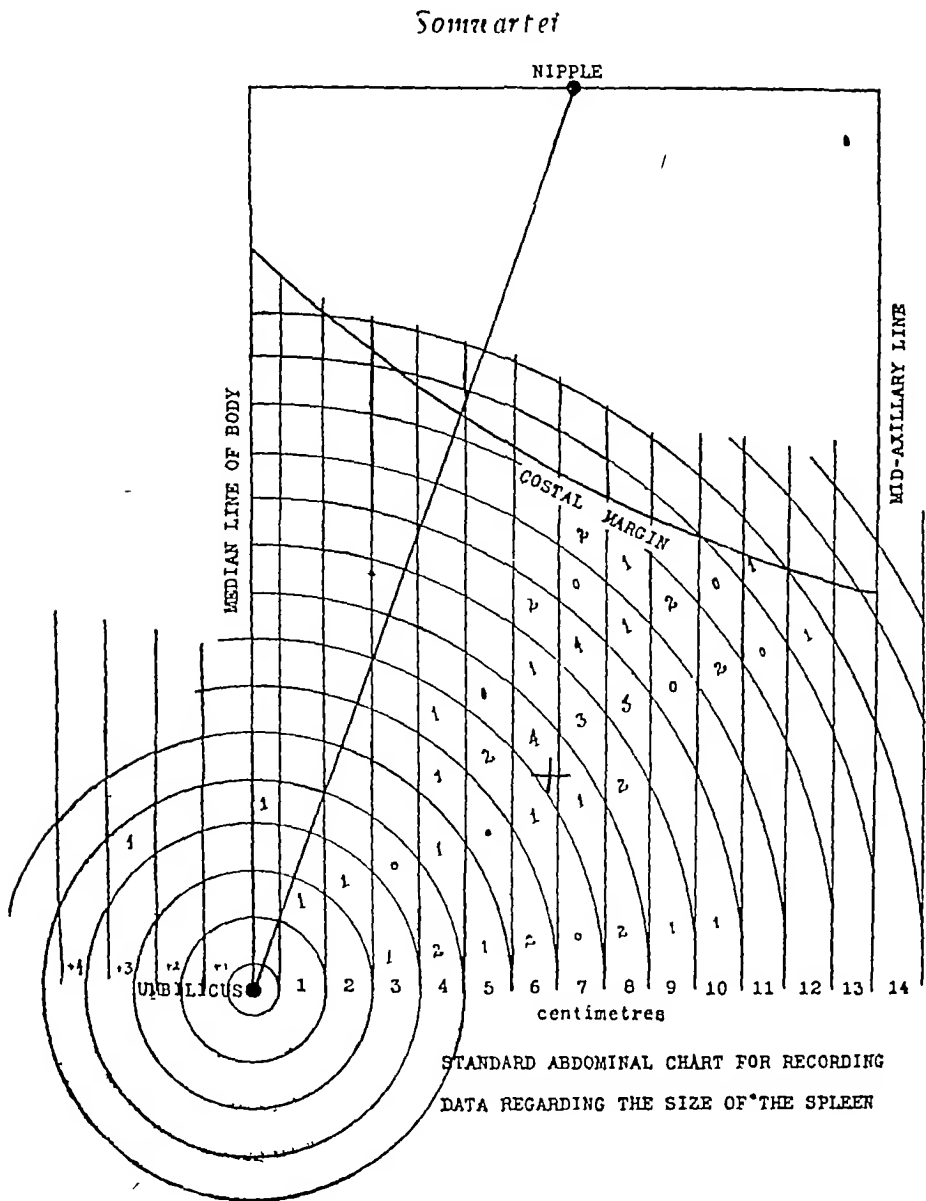


## IV SOMWARPET

In Somwarpet, in North Coorg the examination of 103 children yielded 51 with enlarged spleens, splenic index 49·5 per cent

The corrected average costal projection was 5·9 cms

Only 30 adults were available for examination in the bazar of whom 14 had enlarged spleens, 46·6 per cent



The apex of the average enlarged infantile spleen lay in square 8|6, distant 7·8 cms from the umbilicus, and 4·6 cms above its level. The distance 'apex to middle line' was 6·3 cms

as to whether the fever season is in the autumn, as in Fraserpet, or whether it is in the spring

The vicinity of the stream, which, in the condition of dry weather flow is probably a prolific breeding ground of anophelines, and the fact that two to the labour force were suffering from pyrexia seems to indicate that possibly a spring incidence may have to be reckoned with here

#### SPLENOMETRY

Six hundred and twenty-five children, of all ages up to and including those of ten years, were examined in detail in five different centres. Of these, 406 showed varying degrees of splenic enlargement, yielding a total splenic index for Coorg of 63 per cent

The classification of Coorg with reference to its malariousness is, therefore, that of hyper-endemy, inasmuch as its splenic index is above 50 per cent

The method adopted was that described by Christophers' (1924), namely, measurements of the position of the apex of the enlarged spleen, the exact situation of which is triangulated by measuring its distance from the umbilicus and from the middle line of the body in centimetres. Two other measurements were also taken, viz, the projection of the apex below the costal margin, and the distance between the nipple and the umbilicus. The former of these two affords a comparison with measurements in fingerbreadths, and the latter yields a correction factor whereby the observed figures have been corrected to the scale of the 'standard child' as described by Christophers and Khazan Chand (1924)

This method has been used by Macdonald (1926) in Sierra Leone, and Covell (1927) in the Andamans, and for a detailed account of the method, reference may be made to the original description

These observations were made in Mercara, Verajpet, Gonikoppal, Somwarpet and Fraserpet

The recorded figures, corrected to the scale of the standard child, have been plotted out on Christopher's standard abdominal charts for each of the series of observations

The position of the average spleen in each of the series has been determined by the following method

If its position is determined by merely averaging the two measurements, 'apex to middle line,' and 'apex to umbilicus,' there is a considerable error in evaluating outlying positions of the apex

A correct average can, however, be obtained by a method devised by Lieut-Col S R Christophers, C I E, O B E, F R S, I M S, and demonstrated by him to the senior writer

The method consists in denoting the positions of each apex point as measurements along each of two axes passing at right angles through the umbilicus as zero

One axis, the vertical, is the mid line of the body, and the other is a horizontal line drawn through the umbilicus

The apex of the corrected average infantile spleen lay in square 7|6, its distance from the umbilicus was 7.2 cms, and above its level, 3.9 cms. The distance from the middle line was 6.1 cms.

It should be explained that the measurements were done by the senior author personally, and the age composition in each place was approximately the same, i.e., a random selection of about 20 of each of five age-groups, viz., 1-2 years, 3-4 years, 5-6 years, 7-8 years and 9-10 years, the social classes were the same, and the population from which they were drawn is stable and unaffected by migration. No fallacies arise which are due to a change in the observer or to error in sampling, and the figures are fairly comparable and reasonably accurate.

Tabulated for comparison these figures give the following results —

	Corrected distance from umbilicus (in cms.)	Corrected distance from middle line,	Splenic index (per cent)	Adult spleen rate (per cent)	Corrected costal projection in children (in cms.).	Corrected costal projection in adults (in cms.)
Mercara	7.1	5.6	55.4	53	6.2	5.3
Verajpet	6.3	5.1	82.8	56	6.8	5
Gonikoppal	7.4	6.5	79.3	28	6	
Somwarpet	7.8	6.3	48.5	46.1	5.9	
Fraserpet	7.4	6.1	64.6	48.2	6	6

These figures reveal the fact that despite the difference in the degree of infection, as shown by the splenic index, the size of the corrected average spleen, as measured by its distance from the umbilicus is practically the same in all five places, indeed there is not more than half a fingerbreadth of difference in the position of the apex of the average enlarged spleen in all of them.

It seems legitimate therefore to deduce from the mean of these figures that in Coorg the apex of the average enlarged spleen lies 7.1 cms from the umbilicus and 5.8 cms from the middle line of the body, and that the average costal projection is 6 cms.

This may be compared with Covell's observations in the Andamans where in a community living under hyper-endemic conditions, in 712 adults he found that the mean apex-umbilicus measurement was 6.3, and the mean costal projection was 6 cms and with Macdonald's (1926) in Sierra Leone who showed that among 337 children living in a hyper-endemic area, the average distance of the spleen from the umbilicus was 8.1 cms.

Some observations were also made as to the degree of splenic enlargement in adults.

These were made by seating oneself in a convenient place in the bazar and with local assistance, inducing all passers-by, shopkeepers, coolies, country people etc., to come to see whether or not they had an enlarged spleen.

The cross on the charts marks the position, thus deduced, of the apex of the average spleen of each series

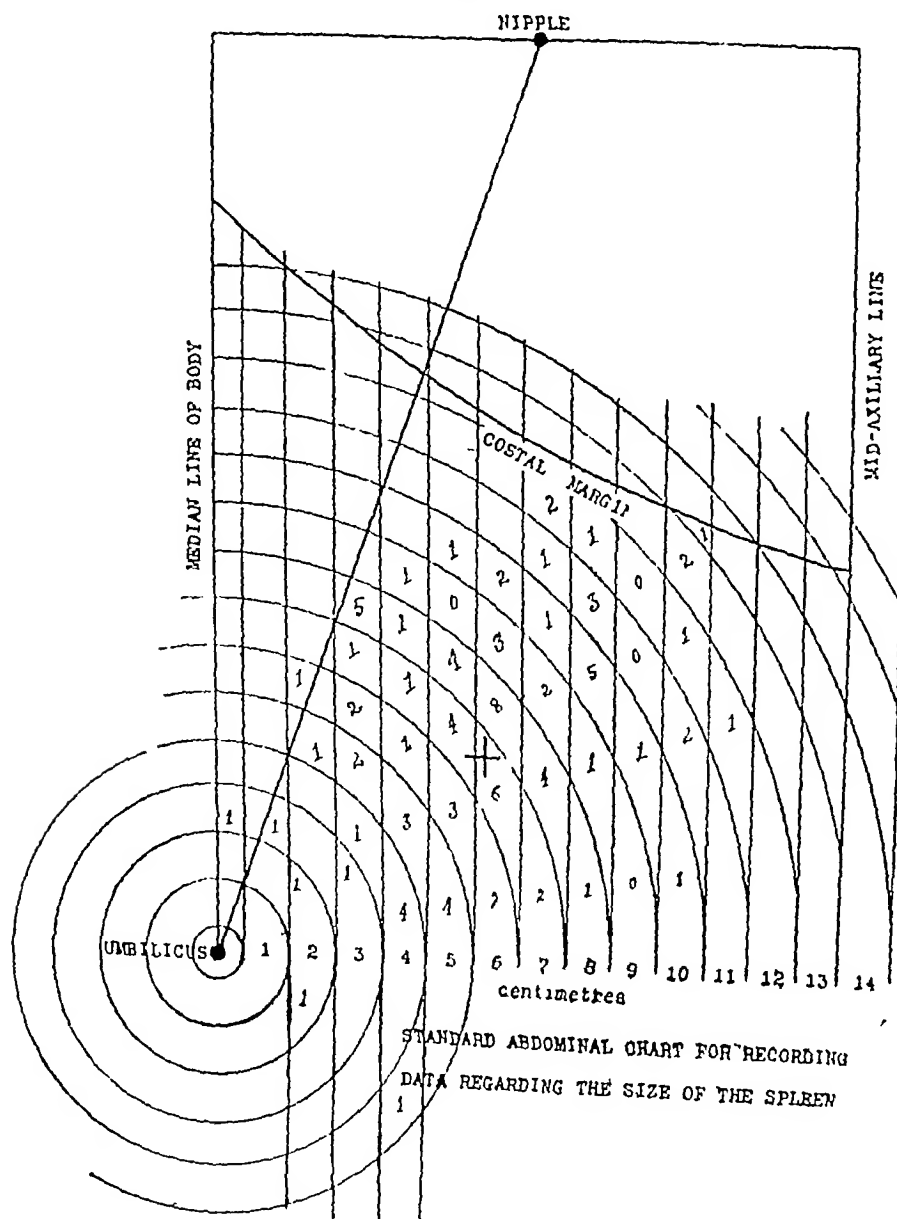
The attached standard charts depict the result of the spleen measurements in children in the five-centies examined, which yielded the following corrected means

### I MIERCARA

Out of a total of 177 children, 98 had enlarged spleens or 55.4 per cent

The corrected average infantile spleen had a costal projection of 6.2 cms from the costal margin (3 fb)

### Miercara



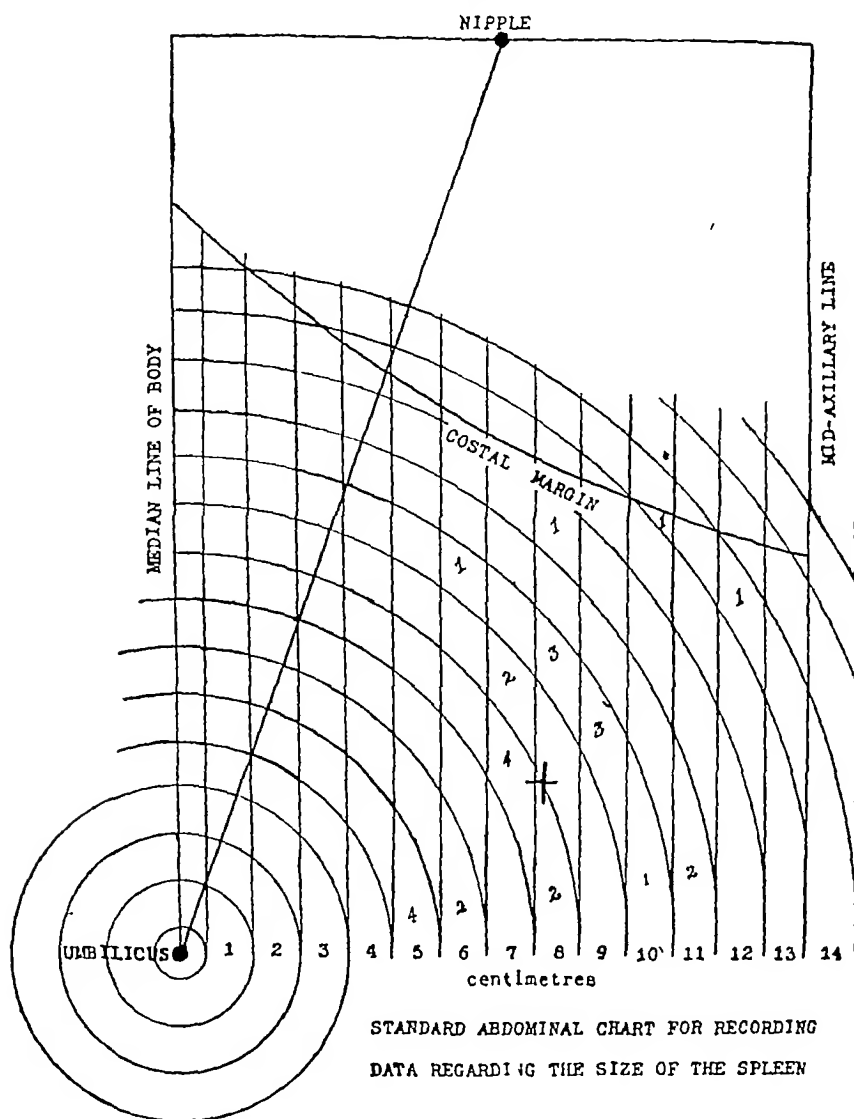
The average adult spleen among 100 bazar adults were 3.3 fingerbreadths or 6.6 cms which corrected to the standard size was 5.5 cms

### SPLEEN ENLARGEMENT BY AGE GROUPS

The above table shows the incidence of splenic enlargement in the age groups under examination

The table shows the rise in the splenic index to its highest point, 76.5 per cent, in the age group 7-8 years, and a commencing fall in the age group 9-10

#### (1) Spleen at one to two years of age



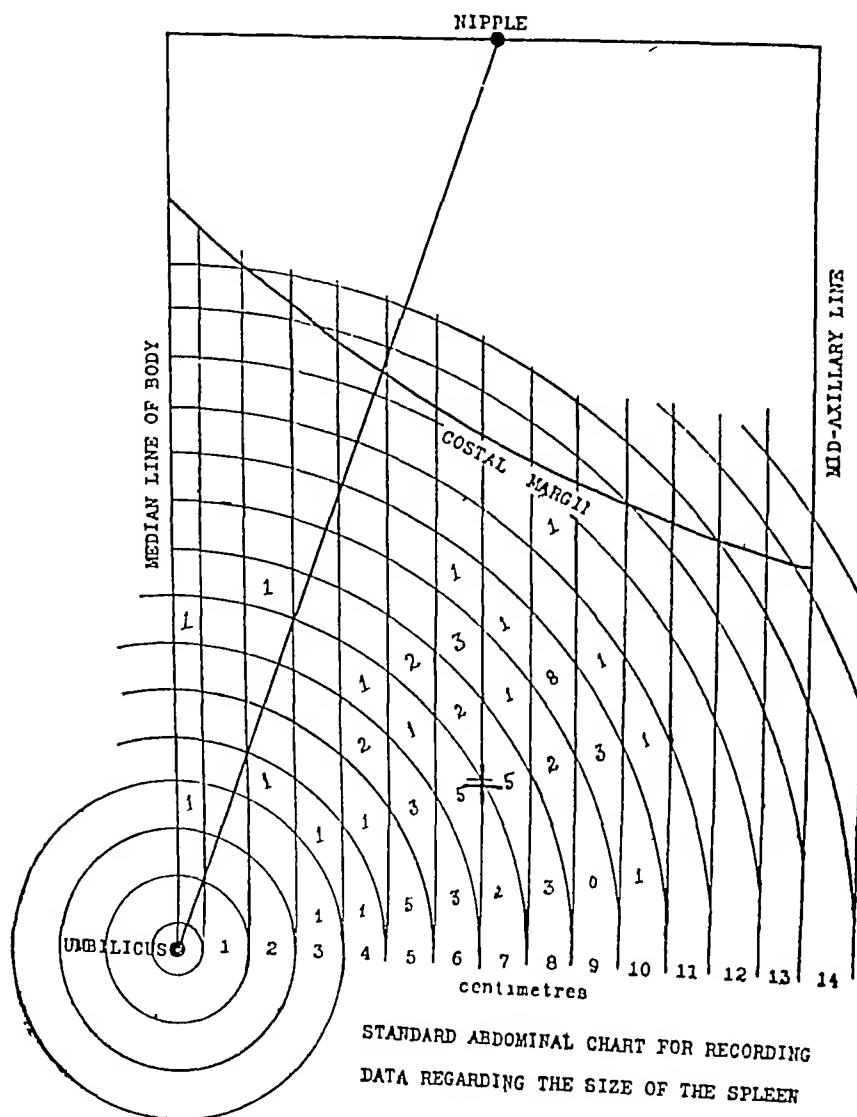
years, with 68.3 per cent. With this may be compared the adult spleen rate in Mercara and Verajpet of 53 and 56 per cent, which indicates to some extent the degree of immunity attained in adult life

## III GONIKOPPAL.

In Gonikoppal, also in South Coorg, among 83 children, 66 had enlarged spleens, or 79.5 per cent splenic index

The corrected infantile average costal projection of the apex was 6 cms

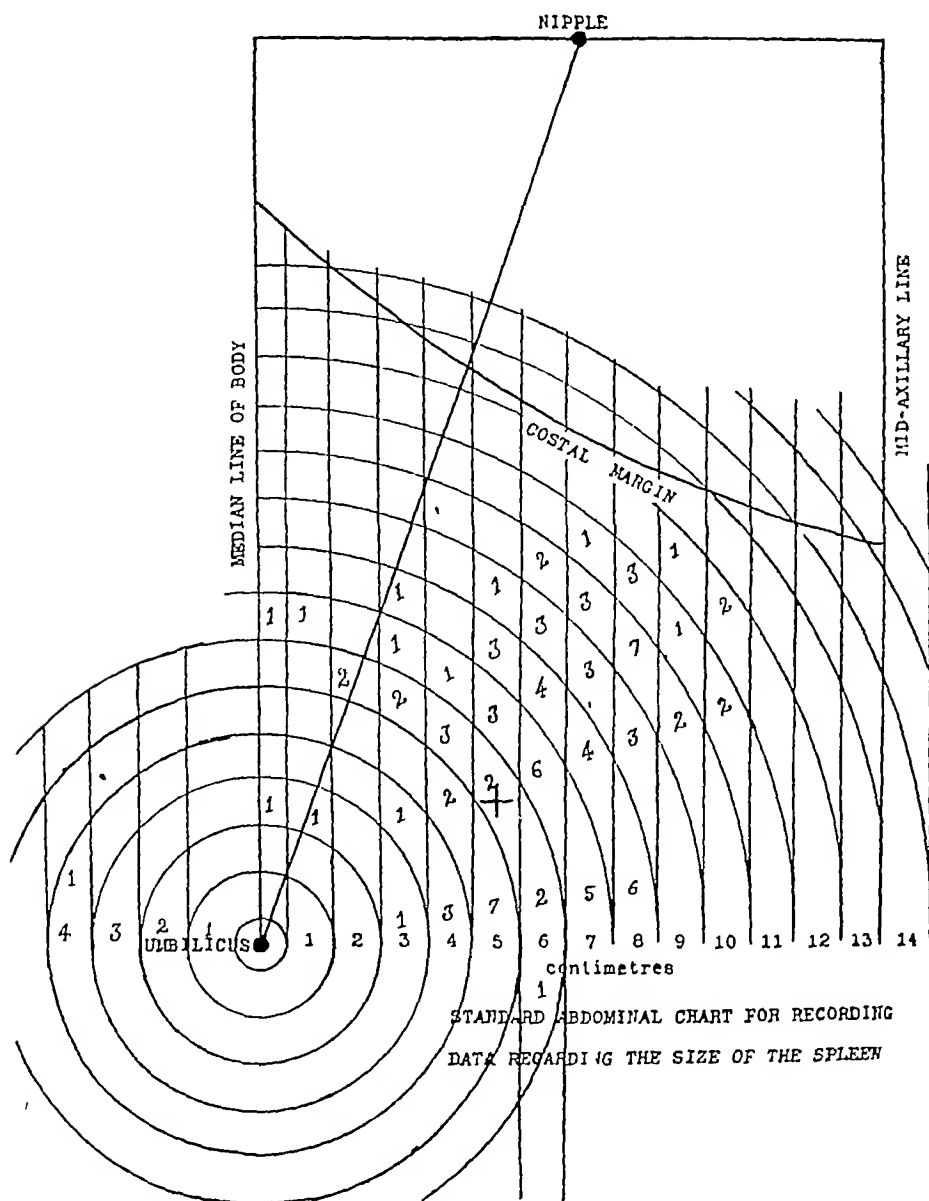
The bazar being small, only 25 adults were examined here, of whom 7 had enlarged spleens, 28 per cent

*Gonikoppal*

The apex of the corrected average infantile spleen lay in square 7|5  
 The distance of the apex from the umbilicus was 7.4, and 3.7 cms above its level. The distance 'apex to middle line' was 6.5 cms

and it was 7.7 cms from the middle line of the body (2) At 3-4 years, we find that the apex has moved 5 cm, nearer the umbilicus, lying now in square 8|7, the measurement being 'apex to umbilicus' 8.3 cms, 'apex above umbilicus' 3.8 cms and 'apex to middle line' 7.4 cms (3) At 5-6 years the apex has moved considerably nearer the umbilicus, lying now in square 6|5, 'apex to umbilicus' 5.9 cms, 'apex above umbilicus' 3 cms and 'apex to middle line' 5.1

(3) *Spleen at five to six years of age*

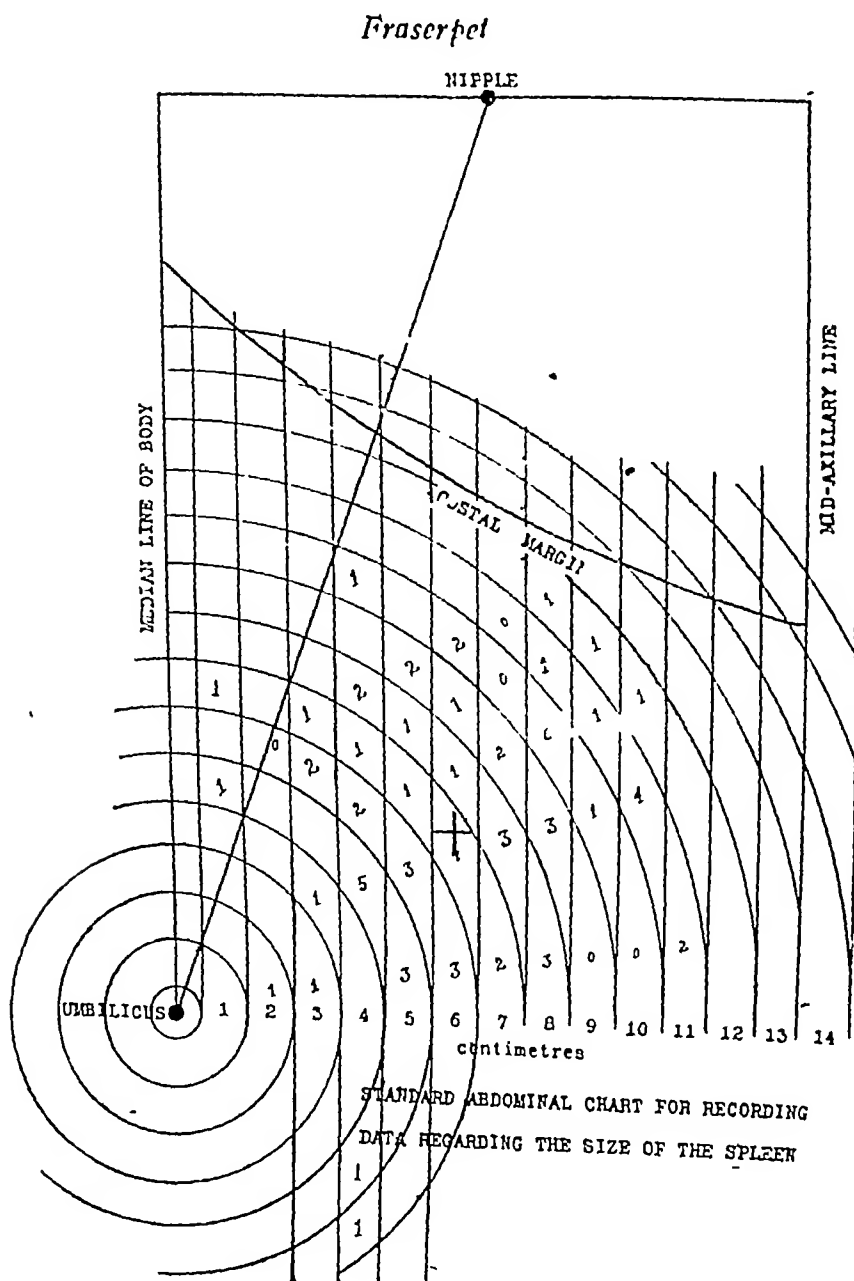


(4) At 7-8 years, the apex has receded a little, lying in square 7|5, measurement 'apex to umbilicus' 6.9 cms and 'above umbilicus' 4.3 cms, 'apex to middle line' 5.4 cms

## V FRASERPET

In Fraserpet, 113 children were examined of whom 73 had enlarged spleens, splenic index 64.6 per cent

The corrected average costal projection among these children was 6 cms  
Of 56 bazar adults, 27, or 48.2 per cent had enlarged spleens



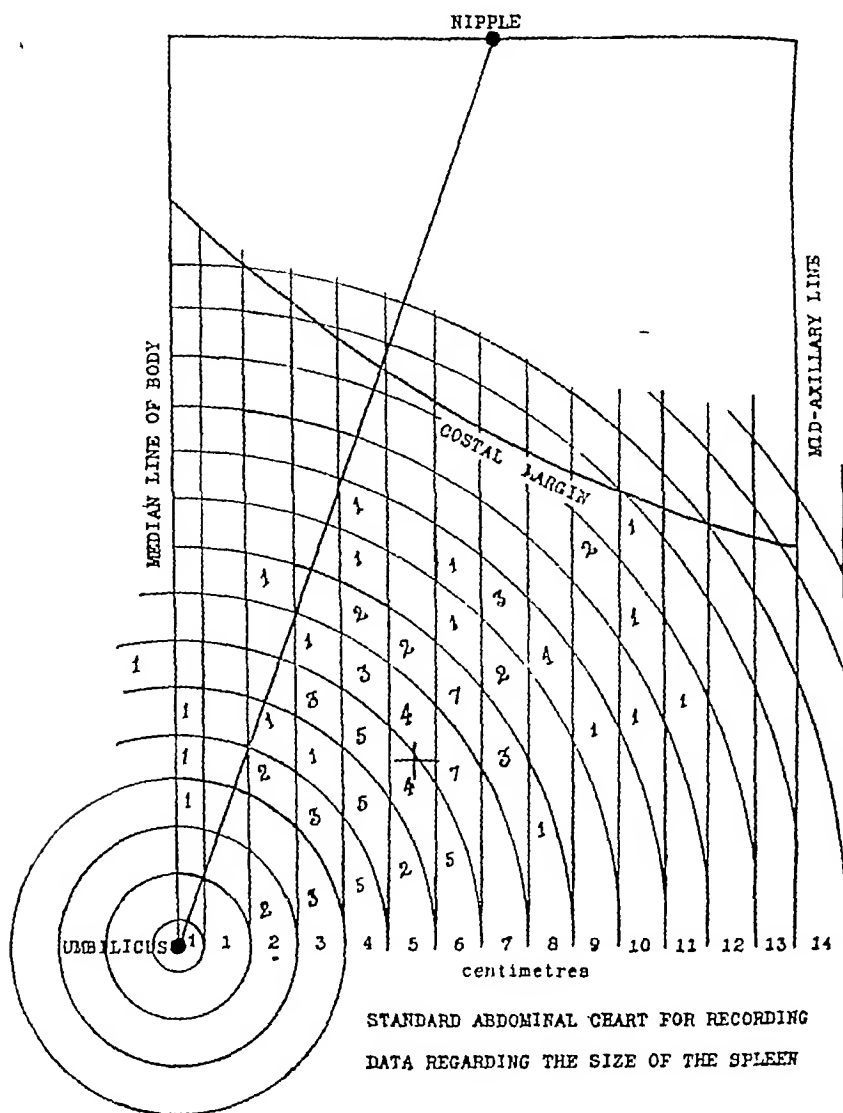
The average degree of splenic enlargement was 3.7 fingerbreadth  $\times 2 = 7.4$  cms or about 6 cms when corrected to the standard scale



The attached chart No 6 shows how the position of the average enlarged spleen of these 5 age groups fall into two different groups

It is difficult to assess the significance of these figures, for it must be admitted that the value of this method of splenometry is still 'sub judice'

(5) *Spleen at nine to ten years of age*



Without attempting any unwarranted deductions, one may simply state (1) that the average spleen in Coorg, a hyper-endemic area, with a stable population, has its apex 7.1 cms from the umbilicus and 5.8 cms from the middle line of the body

This was in all cases tested with the subject in the recumbent position, and the results were noted in fingerbreadths notation. For purposes of comparison with the more precise measurements taken of children, assuming a fingerbreadth to be equal to two centimetres, the recorded adult measurements have been multiplied by two and, like the recorded figures for the measurements of the children's costal projection, they have been reduced to the scale of the standard child.

The size of the average adult enlarged spleen corrected to standard size for comparison was 9 cm less than the infantile enlarged spleen in Mercara and 1.8 cms less in Verajpet, places with a spring incidence.

In Fraserpet with its autumn incidence, in so far as the figures of 56 adult examinations are reliable for comparison, there was no difference between the corrected size of the average enlarged spleen in children and adults.

In Gonikoppal and Somwarpet, the adult observations were too few to warrant inclusion in a comparative total.

*Table showing Splenic Enlargement by Age Groups*

Age	1 to 2 years		3 to 4 years		5 to 6 years		7 to 8 years		9 to 10 years	
	+	—	+	—	+	—	+	—	+	—
Mercara	4	8	13	12	22	25	34	17	25	17
Somwarpet	4	15	10	13	13	9	11	9	11	12
Gonikoppal	11	3	9	3	20	3	12	4	14	3
Fraserpet	2	7	5	10	29	5	22	6	15	11
Verajpet	7	10	8	4	15	5	58	6	32	2
TOTAL	28	43	45	42	99	47	137	42	97	45
Percentage positives	39.4		51.7		67.7		76.5		68.3	

NOTE Total observations

625

Positive

406

Splenic index for Coorg

63.3 per cent

One of us (J D B), was the operator in all cases, and the method of collection was therefore undisturbed by any variation due to difference in technique

Owing to the humid atmosphere of Mercara, special measures were required to preserve the slides for future examination

They were stained the day after collection, and when dry, were put up in parcels of 1 dozen (each slide separated from the other by a binding string) They were then packed in a large cheroot drying tin with an absorbent pad in its lower compartment

By this method, exposure to an atmosphere with a 90 per cent humidity, and the rapid deterioration of the stained films which otherwise would have occurred, was successfully obviated

In counting, the number of parasites present in fields showing a total of 500 fowl cells was enumerated. A negative result is therefore the result of examination of that number of fields which yielded 500 fowl cells but no malaria parasites

The following is an analysis of the result of the examination of 540 such blood slides taken in five different places in Coorg

TABLE I

	Number with parasites	Number examined	Endemic index (per cent)
Mercara	77	177	43.5
Verajpet	61	128	47.6
Fraserpet	13	60	21.6
Gonikoppal	37	83	44.6
Somwarpet	16	92	16.1
Total, Coorg	204	540	37.7

The endemic index for Coorg on the percentage of children showing malarial parasites in their blood, was 37.7 per cent

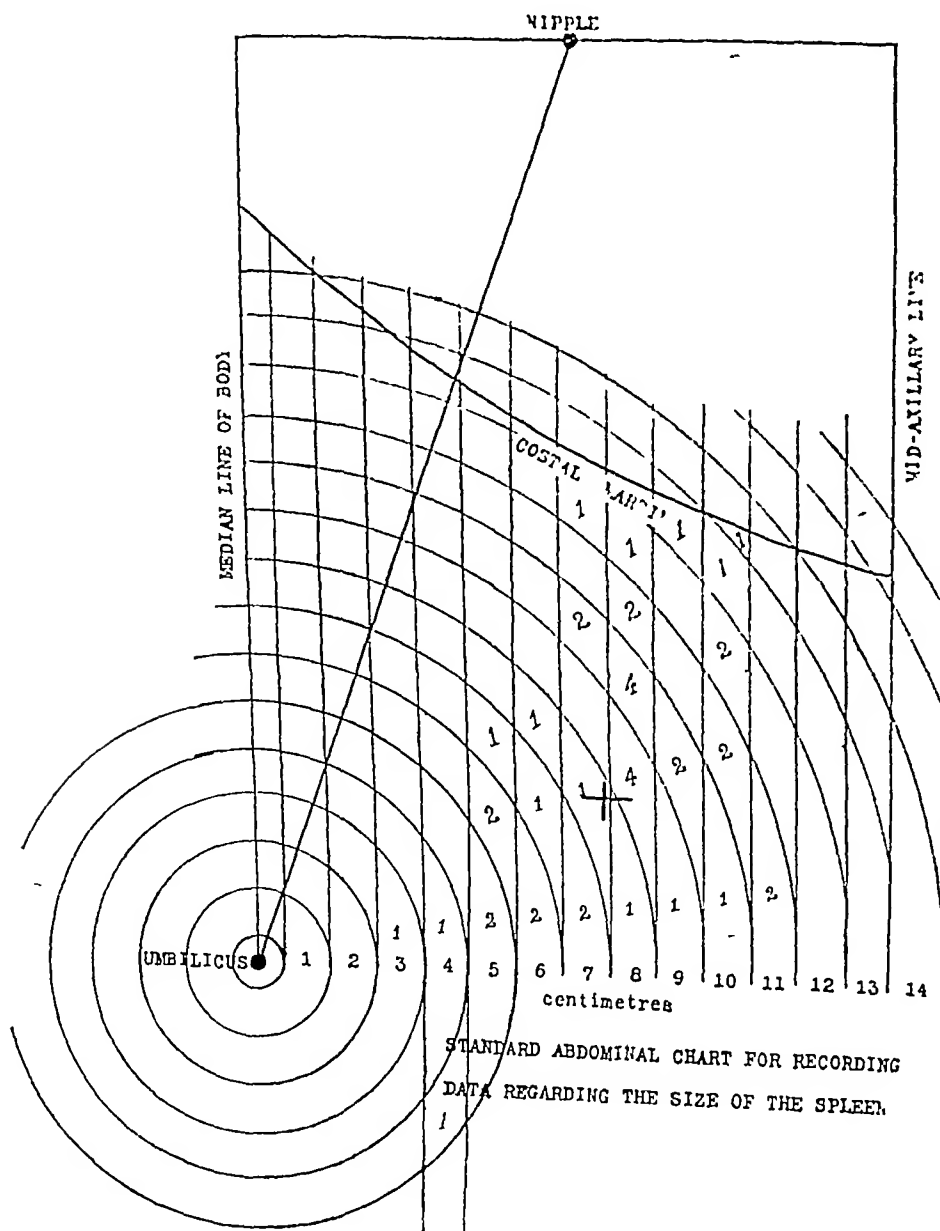
Verajpet yielded the highest endemic index 47.6 per cent, followed by Gonikoppal 44.6 per cent and Mercara 43.5 per cent

Table II shows the proportions of the different species of malaria parasites, and of gametocyte infections reckoned in terms of crescents. From this table it will be seen that if crescents and mixed infections are added to *P. falciparum* infections, more than half the infections in Coorg are due to *P. falciparum*, *P. vivax* and *P. malariae* are in about equal proportions, and mixed infections are 6 per cent throughout. Mercara has the highest percentage of

If these figures be analysed from the point of view of the size of the enlarged spleen at different ages, the following are the results

Standard charts have been prepared showing the corrected position of the apex of the enlarged spleens observed in the age group 1-2 years, 3-4 years, 5-6 years and 9-10 years and the position of the average enlarged spleen in each age group has been calculated by the method already described

(2) *Spleen at three to four years of age.*



In age group (1) 1-2, the apex of the average enlarged spleen lay in square 9|8, its distance from the umbilicus was 8.8 cms and 3.7 cms vertically above it,  
J, MR

Table III shows the parasite value per infection in each of the five places under observation. It shows a very active infection in Gonikoppal due to *P. falciparum* and *P. malariae*, there the average parasite value per infection (9,735) is above that of 5,000 per cm, which is reckoned as representing attack conditions (Christophers, 1924)

TABLE III

Place	<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	MIXED INFECTION			Cres cents	Average parasite value per infection
				<i>P. vivax</i> and <i>P. falciparum</i>	<i>P. vivax</i> and <i>P. malariae</i>	<i>P. malariae</i> and <i>P. falciparum</i>		
TOTAL	106 400	26 328	5,444	7,338	816	1 320	2,808	
Mercara								
Average per infection	2 875 3	1,548 7	340 2	1 467	16	1 320	165	1 955
TOTAL	31,224	13,984	1,704	14,192		96	2,784	
Verajpet								
Average per infection	1,248 8	690 2	243 3	4,730 7		96	307	1,049
TOTAL	312	3,308	2,452		144			
Fraserpet								
Average per infection	104	1,654	350 3		144			478
TOTAL	27 110	1,488	75,984		4 243		240	
Gonikoppal								
Average per infection	1,936 5	372	6 907		4,243		48	9,735
TOTAL	264	1 712	924			1,888	48	
Somwarpet								
Average per infection	528	570 6	154	.		1,888	48	302

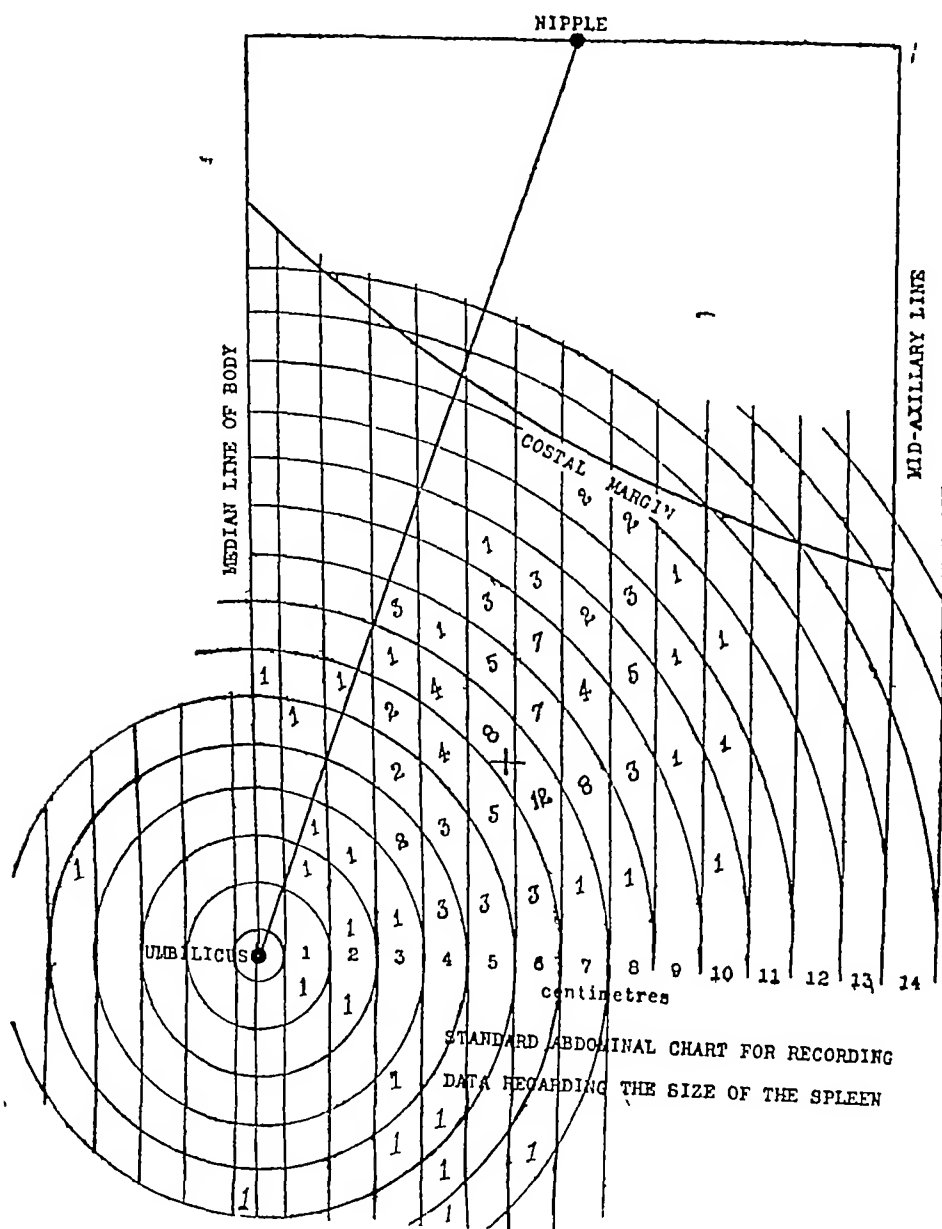
To economise space the actual counts are not detailed, but out of 14 *P. falciparum* infections, four gave the following counts in excess of 5,000 per cm, viz, 125,520 18,600, 106,680, 13 320, and out of the eleven *P. malariae* infections, one gave more than 5,000 per cm, viz, 70,464

In Mercara there is also evidence of an infection only a little less active. Six of the 37 *P. falciparum* infections are at, or above 'attack' numbers, viz, 10 176 44,800, 25,100, 8,880 and 4,920 and one of the 17 *P. vivax* infections, viz, 22,800. In Verajpet, the average parasite value per infection is lower: two out of 25 *P. falciparum* infections were above attack value, viz, 16,392 and 7,300. The infection here seems to have been one in which the spring incidence was

One notices that this chart contains practically all the very large spleens whose apices descend below the umbilicus, or reach, or cross the middle line of the body \*

(5) At 9-10 years, the apex lies again in square 6|5, it is 6.4 cms from the umbilicus and 4.0 cms above its level. Its distance from the middle line of the body is 5 cms

(4) *Spleen at seven to eight years of age*



\* Note—The recession of the apex in the 7-8 years age group, followed by an advance again at 9-10, is curious, and it may be observed that Macdonald (1926) found the same thing in this age group of his hyper-endemic series

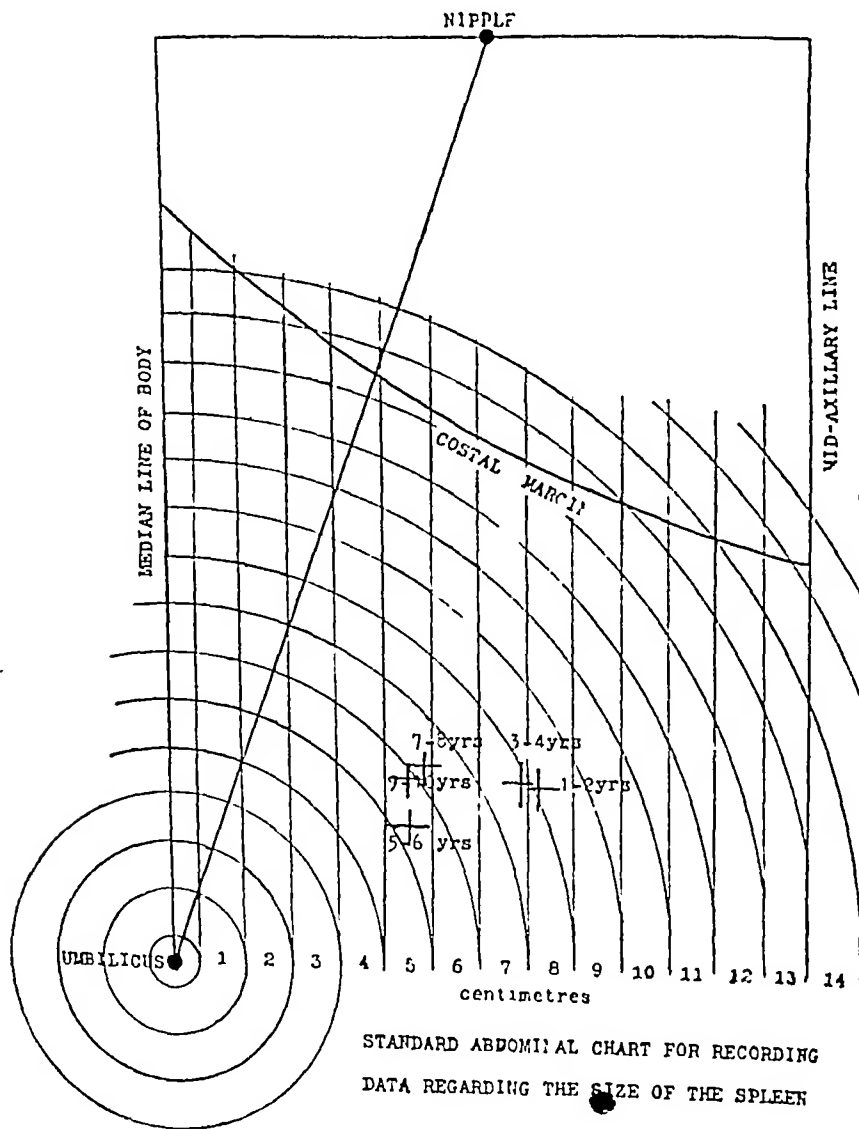
TABLE IV—(contd)

Place	Number	Number infected	Percentage infected	Parasite count	Total parasites	Average parasites per cm per unit of group
<i>Age Group 5 6 Years</i>						
Mercara	47	20	42.6	24, 816, 24, 96, 48, 144, 672, 240, 144, 72, 432, 96, 72, 1, 320, 360, 48, 960, 792, 648, 264	7, 272	154.7
Verajpet	20	7	35.1	480, 816, 428, 120, 12, 912, 24, 120	14, 900	745
Fraserpet	34	5	14.7	24, 1, 392, 24, 264, 144	1, 848	54
Gonikoppal	23	13	56.5	24, 24, 1, 176, 816, 216, 48, 5, 496, 240, 96, 508, 144, 3, 600, 48	12, 436	540.7
Somwarpet	22	6	27.3	24, 48, 800, 24, 144, 72	1, 112	50.5
TOTAL	146	51	28		37, 568	257.3
<i>Age Group 7 8 Years</i>						
Mercara	51	21	41.2	48, 48, 72, 240, 216, 168, 792, 1, 104, 24, 24, 2, 880, 96, 24, 24, 25, 100, 72, 1, 056, 2, 736, 48, 240, 48, 24	35, 060	687.4
Verajpet	64	28	43.7	96, 1, 272, 48, 864, 48, 168, 4, 608, 120, 528, 336, 72, 1, 080, 192, 216, 120, 72, 960, 144, 96, 312, 1, 960, 48, 120, 2, 184, 72, 48, 936, 72	16, 782	262.2
Fraserpet	28	5	18	528, 48, 120, 24, 72	792	29
Gonikoppal	16	5	31	106, 680, 1, 128, 1, 416, 24, 13, 320	122, 628	761.2
Somwarpet	20	3	15	48, 120, 1, 888	2, 056	102.8
TOTAL	179	62	34		177, 318	990.6

(2) The average spleens fall into two groups

(i) From 1—4 years, the average enlarged spleen has its apex about 8.5 cms from the umbilicus and is about 7.5 cms from the middle line.

(6) Chart to show grouping of average spleen at different age groups



(n) From 5—10 years, the position of the apex of the spleen is from 6 to 7 cms from the umbilicus, and about 5 cms from the middle line

## BLOOD COUNTS AND PARASITE RATES

The method employed was that described by Sinton (1924) using a standard suspension of fowl corpuscles

An improvement in technique was employed, suggested by Major J A Sinton, VC, OBE, I MS, by which vaccine capillary tubes replaced capillary pipettes, which are fragile and not conveniently portable.



Gonikoppal with a parasite value of 13,423 in the age group 1-2 years admirably illustrates 'acute infestation'. Its progress toward some degree of immunity is well seen in the parasite counts in the succeeding age groups

1-2 years	13,423
3-4 "	1,810
5-6 "	540
7-8 "	764
9-10 "	918

The following table display these relationships —

TABLE V  
*Relation between Splenic Measurement and Parasite Counts*

Place	Observations	Total positives	Percentage positives	Parasite count	Total parasites	Average parasites per unit of group
Negative Spleen						
Mercara	79	20	20.4	24, 816, 24, 784, 144, 672, 24, 24, 24, 24, 10, 176, 24, 72, 48, 96, 504, 48, 816, 888, 24	15,456	208
Verajpet	22	4	18.2	480, 120, 2,184, 72	2,856	129.8
Fraserpet	40	3	7.5	140, 144, 240	524	13
Gonikoppal	17	10	58.8	48, 216, 216, 3,600, 144, 3,900, 508, 72, 13,320	22,024	1,295.5
Somwarpet	53	6	11.3	840, 60, 48, 24, 24, 72	1,068	20
TOTAL	211	43	20		41,028	105
Small Spleens 11 to 15 cm*						
Mercara	14	8	57	240, 10, 176, 216, 291, 960, 528, 8,880, 1,176	22,467	1,604.8
Verajpet	8	2	25	120, 1,080	1,120	110
Fraserpet	3	2	66	24, 24	48	16
Gonikoppal	3	2	66	120, 24	144	48
Somwarpet	10					
TOTAL	38	14	36.8		23,779	626

*P. falciparum* infections (48 per cent), Fraserpet has a relatively high percentage of *P. malariae* infections, but a low endemic index. As we have seen, its season of malarial prevalence is in the autumn and not the spring, at the time of our observations nearly a year had elapsed since its last malaria season, and it appears that we are dealing here with a residual infection in which the quartan parasite, notoriously difficult to eradicate, has survived disproportionately.

TABLE II

Places		<i>P. falciparum</i>	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. vivax</i> and <i>P. falciparum</i>	<i>P. vivax</i> and <i>P. malariae</i>	<i>P. malariae</i> and <i>P. falciparum</i>	Crescents
Mercara	No	37	17	16	5	1	1	17
	per cent	48	22	20.7		9		22
Verajpet	No	25	20	7	3		1	7
	per cent	41	32.8	11.4		6		11.4
Fraserpet	No	3	2	8				
	per cent	23.9	15.4	61.5				
Gonikoppal	No	14	4	11		1	2	5
	per cent	38	10.8	30		8.1		13.5
Somwarpet	No	5	3	6			1	1
	per cent	31.2	18.8	37.5		6		6
Coorg	No	84	46	48	15			30
	per cent	41.1	22.5	23	6.2			14.6

## CRESCENTS

Crescent carriers are 14.6 per cent of the total infections.

The proportion is highest in Mercara, with an active infection in which *P. falciparum* predominates, and is fairly high in Gonikoppal which, as we shall see later, had an acute and active infection at the time it was seen. Fraserpet, with its residual infection yielded no crescents, and Somwarpet with a low endemic index and an inactive infection, yielded only one crescent (6 per cent).

This tallies with the view advanced by Schuffner (1920) and Christophers (1924) as to the production of crescents being associated with an acute infection rather than with immune infestation.

TABLE V—(concl'd)

Place	Observations	Total positives	Percentage positives	Parasite count	Total parasites	Average parasites per unit of group
Very Large Spleens 4 cms and less						
Mercara	13	6	46.1	168, 96, 96, 2,736, 72, 264	3,432	264.0
Verajpet	17	7	41.2	336, 1,960, 96, 72, 72, 24, 72	2,632	154.8
Fraserpet	3	1		96	96	32
Gonikoppal	11	5	36.3	24, 120, 360, 144	748	68
Somwarpot	6	3		120, 360, 144	524	87.3
TOTAL	50	22	44		7,432	148.6

(a) *Negative Spleens*—Twenty per cent of the children with no splenic enlargement showed parasites in their blood, and the average parasite value of the group was 195

The acute infestation in Gonikoppal has a parasite value of 1,293.5, and obscures the low value for the other four places

(b) *Enlarged Spleens*—Those showing splenic enlargement have been grouped into four classes—

1 Small spleens, 11—15 cms distant from the umbilicus (1-2 fingerbreadths)

2 Medium spleens 8—10 cms distant (3 fingerbreadths)

3 Large spleen 5—7 cms from the umbilicus (4-5 fingerbreadths)

4 Very large reaching umbilicus, or beyond it

I The Small Spleens, of which 36.8 per cent show parasite infection, have an average parasite value of 626

In Mercara, the parasite value in this group is large

II The Medium Spleens, 8—10 cms from the umbilicus, are the group in which, as we have seen, lies the average spleen of the age groups 1-2 years and 3-4 years

The percentage of this group which shows parasites in the blood is 37.3 per cent

The acute infection of Gonikoppal yields a high average parasite value of 6,491 and the average parasite value for the group is 2,135

III The Large Spleens, 5—7 cms from the umbilicus, belong to the group in which lies the average spleen of the age groups from five to ten years of age, and is the group in which lies the average spleen of all places examined

earlier or terminated sooner, on judging from its higher spleen index of 82.8 per cent, it is one in which immunity is better developed than it is in Mercara.

The high parasite rates evidence of much active infection, with the comparatively low spleen rate in Mercara seem to suggest an active and progressive infection, and indicates that there is some ground for the suspicion that malaria is on the increase in that town.

#### PARASITE INDEX BY AGE GROUPS (Table IV)

In the age group 1 to 2 years the percentage showing parasites is 27.6 per cent. The different places show very different results, thus in Gonikoppal

TABLE IV

Place	Number	Number infected	Percentage infected	Parasite count	Total parasites	Average parasites per cent per unit of group
<i>Age Group 1-2 Years</i>						
Mercara	12	3	25	912, 216, 72	1,200	100
Verajpet	17	2	11.8	108, 24	190	11.1
Fraserpet						
Gonikoppal	14	9	64.4	125, 520, 4, 248, 48, 504, 144, 912, 70, 464, 48, 24	201,922	13,423
Somwarpet	19	3	15.8	60, 48, 264	372	19.5
TOTAL	62	17	27.6		203,684	3,285.2
<i>Age Group 3-4 Years</i>						
Mercara	25	12	48	291, 22, 800, 960, 144, 528, 504, 720, 1, 656, 8, 880, 1, 170, 888, 24	38,565	1,542.5
Verajpet	12	4	33.3	192, 1, 152, 416, 24	1,784	148.6
Fraserpet	12	2	16.6	240, 3, 236	3,476	289.6
Gonikoppal	12	7	58.3	120, 24, 216, 18, 600, 216, 2, 400, 144	21,720	1,810
Somwarpet	23	3	13	840, 360, 24	1,224	53.2
TOTAL	84	28	33.3		66,769	794.7

*A philippinensis*—In a clear water tank situated in coffee cultivation

*A karwari*—Found with *A maculipalpis* in jungle streams

*A maculatus*—With *A listoni* (one specimen only)

*A aitkeni*—In a shady stream and in a rocky drainage channel

*A tessalatus*—With *A aitkeni*, in a rocky drainage channel

*A jamesi*—In hill streams and channels in swampy valleys

The adult bag was very small, despite frequent search in houses. The predominant anopheles in Coorg probably shelter in jungle and not in houses, and heavy and constant rain prevented our searching for them in jungle.

*A listoni* was collected in small numbers in houses in Mercara and Verajpet.

*A fuliginosus* and *A karwari* were found in a cowshed in Verajpet, *A tessalatus* and *A jeyportsiensis* were found in a stable at Hallery and *A vagus* in a cowshed in Fraserpet.

*Phlebotominae*—Although adult mosquitoes were few and far between in the houses which we searched, it was otherwise with sandflies.

Everywhere in Coorg invariably in suitable cowsheds, sometimes in dwelling houses, and once inside what was almost the only mosquito net seen in Coorg houses, specimens of *P argentipes* were collected, sometimes in large numbers.

In one instance, in a coffee planter's bungalow in South Coorg, they were so numerous as to constitute a house pest.

This observation turned our thoughts towards the possibility that Kala-azar might be found in Coorg.

An examination of the dispensary records showed that one case of Kala-azar was recorded in 1910-11, another in 1912-13 and five in 1921-22.

As far as could be ascertained, these cases were believed to have acquired their infection elsewhere than in Coorg, and nowhere in the province could any suspicious history be elicited of the occurrence of a disease associated with splenic enlargement and fever with a distinct house or family incidence, and almost always fatal in about two years.

There is no reason to believe that the malarial problems of Coorg are complicated by the unsuspected prevalence of Kala-azar, but in this connection the temperature and humidity statistics are worth examination as they are not without some significance.

The senior author (McCombie Young, 1924) has had occasion to infer from a study of the distribution of Kala-azar in India that the conditions of temperature and humidity which are required for the transmission of this disease are something as follows:

High maximum summer temperatures, and a winter mean monthly minimum temperature falling below 50°F, such as are found in the dry climates of Northern India, are unfavourable.

What seems to be required is a considerable degree of humidity, not less than is indicated by an annual mean relative humidity of 60 per cent, a monthly mean relative humidity not at any time less than 40 per cent to 50 per cent, and a mean monthly minimum temperature never less than 50°F.

The following table shows that Mercara, which is climatically typical of the greater part of Coorg, has an annual mean relative humidity of 80 per cent, a

TABLE IV—(concl'd)

Place	Number	Number infected	Percentage infected	Parasite count	Total parasites	Average parasites per cm per unit of group
<i>Age Group 9 10 Years</i>						
Mercara	42	16	38	21, 00, 72, 141, 10 170, 24, 44, 800, 110, 00, 210, 2, 448, 4, 920, 210, 24, 24, 216	03, 006	1,514 4
Verajpet	34	19	55 9	480, 108, 480, 144, 16, 302, 264, 72, 00, 360, 72, 3 000, 204, 24, 48, 48, 216, 48, 48, 7, 300	30, 220	888 8
Fraserpet	26	1	3 8	96	96	4 1
Gonikoppal	17	3	17 6	384, 1,104, 72	1 560	91 8
Somwarpet	23	1	4 3	72	72	3 1
TOTAL	142	40	28 1		95 554	673

(active infection) 64 4 per cent of this age group are infected and the average parasite value per unit of group is 3,285 2, whereas in Fraserpet (immune infection), the figures are zero for both

The high parasite value for Gonikoppal obscures the low average in the age group for the other four places, for which, excluding Gonikoppal, it is only 18 4

In the age group 3-4 Mercara now shows signs of an infection presumably less recent than that of Gonikoppal, which still holds the highest place for parasite values

The group shows a percentage infection of 33 3 per cent and has an average parasite value of 794 7

*Age 5-6 years*—The percentage of infection in this group is 28 per cent and the parasite rates in Mercara and Gonikoppal have diminished

*Age 7-8 years*—The percentage infection of group is 34 per cent and the average parasite value is 990

*Age 9-10 years*—The average parasite value of the group is 673 and the percentage infection is 28 per cent

Mercara and Verajpet both show high average parasite rates, probably because of the preponderance of *P. falciparum* infections

The consistently low counts in Fraserpet at all ages confirm the indications of the previous analyses that this area, when seen, was in the condition of immune infestation

TABLE V—(contd.)

Place	Observations	Total positives	Percentage positives	Parasite count	Total parasites	Average parasites per unit of group
Medium Spleens 8 to 10 cms						
Mercara	30	10	44.4	48, 96, 18, 18, 72, 2, 160, 11, 04 240, 90, 25, 100, 210, 132, 240, 24, 24, 216	10,164	838
Verajpet	31	10	51.6	1,272, 816, 48, 72, 216, 72, 48, 168, 480, 16, 392 264, 48, 936 120, 216, 24	21,192	683.6
Fraserpet	13	4	30.8	528, 72, 240 3,236	4,076	313.5
Gonikoppal	34	16	47	24, 24, 125, 520 4,248, 504, 144, 912, 70, 164, 10,668, 816, 2,400, 48, 72, 144, 1,416, 13,320	230,724	6,491.8
Somwarpet	20	4	20	72, 264, 48, 24	408	20.4
TOTAL	134	50	37.3	-	286,204	2,135.1

Large Spleens 5 to 7 cms

Mercara	31	9	27.7	24, 2,880, 144, 1,050, 2,448, 768, 72, 102, 44, 800	11,464	369.8
Verajpet	49	27	53.6	96, 48, 168, 428, 4,008, 528, 192, 120, 960, 144, 96, 312, 480, 72, 360, 3,696, 264, 48, 48, 48, 48, 7,300, 168, 192, 416, 12, 912, 24	33,776	689.2
Fraserpet	13	2	14.5	48, 264	312	24
Gonikoppal	24	10	41.7	240, 1,104, 5,400, 48, 1,176, 1,128, 48, 18,600, 216, 384	28,440	1,185
Somwarpet	8	3	37.5	48, 1,888, 800	2,736	342
TOTAL	125	51	40.8	-	76,728	613.8

The percentage of this group which contained parasites was 40.8 per cent and the average parasite value was 613.8

IV In the group of very large spleens, 4 cms from the umbilicus or less, including those reaching the umbilicus or extending below it, 44 per cent of these showed parasites, and the average parasite value was 148.6

The figures for Gonikoppal illustrate the sequence of parasite values in acute infestation conditions

	Parasite value
No spleen enlargement	1,295
Small spleen 11—15 cms	48 (3 only)
Medium	6,491
Large	1,185
Very large	68 (4 only)

*Conclusions*—An analysis of these figures brings one to the same conclusion as that of Christophers (1924), viz., that the highest parasite value is associated with spleens of such a size that the apex lies 8 to 10 cms from the umbilicus, which is, as we have seen, the position of the apex for the first four years of life

These as the age group analysis has shown, are the years of acute parasite infection

The application of these methods of splenometry and quantitative parasite counts in Coorg appears to confirm Christophers' deductions as to the mechanism of immunity, as seen elsewhere. At the same time it may be advanced that they afford useful information as to the state of affairs in different places, which would not emerge from the easier and quicker method of fingerbreadth measurements and qualitative blood examinations

#### ENTOMOLOGICAL

As has been indicated elsewhere, the time of our visit, which almost synchronised with the onset of the monsoon, was unfavourable for such observations, as the heavy rains wash away both larvae and adult mosquitoes

The following species of anopheles were collected as larvae

They are tabulated in what was approximately their order of prevalence

*A. listoni*—This species was found in profusion in open surface drains containing a small stream of water, preferably slightly contaminated with household sullage water

*A. maculipalpis*—Found in running water, in seepage water, in a swamp, and in tanks. Less domestic than *A. listoni* in its breeding grounds, but common in natural waters in Coorg

*A. culicifacies*—In muddy stagnant water, in surface water, in drains, and in rocky pools

*A. minimus*—In occasional association with *A. maculipalpis*

*A. jeyporiensis*—In undrained natural swampy land

*A. vagus*—In stagnant muddy pools, surface drains, and tanks

*A. subpictus*—In overflow water from a tank